Demonstrating the Effect of Trees for Controlling Particulate Matter, Ammonia, and Odor from Poultry Buildings

FINAL PROJECT REPORT

Prepared for:
United States Department of Agriculture - Natural Resources Conservation Service

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Deliverables

1. A tested vegetative shelterbelt system
2. Paper and presentation to one state or national conference on the results of the study
3. Hardcopy of information bulletin on the results of the study intended for poultry producers. Web-based information on the study will also be made available through the College of Forestry and Agriculture website.
4. Cost analysis of the vegetative shelterbelt

December 30, 2016

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EXECUTIVE SUMMARY

Poultry houses generate significant amount of particulate matter (PM), ammonia, (NH_3) hydrogen sulfide (H_2S), and other odorous gases that are expelled to the outside environment through the exhaust fans in the buildings. These PM and gaseous emissions have been known to pose significant health and environmental concerns especially when the buildings are located close to residential, commercial, and recreational areas. Odorous gases trigger complaints from neighbors often resulting in lawsuits, moratoria on expansion of farms, and odor control regulations in some states. Although much current dissatisfaction is driven by concerns about odors, air quality concerns have also focused attention on nitrogen deposition and ammonia emissions for which poultry buildings and poultry manure application are sources. A variety of strategies and control technologies are available for reducing emissions and dispersion of pollutants from animal structures. One control strategy that is natural and is receiving increasing attention is the use of vegetative shelterbelts (or vegetative environmental buffer) to capture farm emissions.

Vegetative shelterbelts are rows of trees and other vegetation planted around poultry buildings or strategically located directly opposite of exhaust fans. Vegetation that are correctly placed will not only create visual barrier or provide shadings that could result in cooler microclimate during summer, but also provide filtration surfaces for PM and gases. When PM and gases are captured on plant surfaces, fewer pollutants will be picked up by the wind passing over the building. Consequently, the impact on the environment and human health and welfare is lessened by collecting and dispersing the pollutants before they reach the nearby community.

This project utilized six different plant species (Arborvitae, Roughleaf dogwood, Eastern red cedar, Yaupon, American holly, and Arizona cypress) that were established in the zone directly opposite of the exhaust fans of four poultry buildings at SFASU's Broiler Research Center. The major findings of this study were the following:

- All six plant species exhibited tolerance to poultry building emissions. Yaupon and Roughleaf dogwood that were planted in the first row and received the most amount of pollution had survival rates of over 90%. Although Arborvitae and Arizona cypress, which were planted in the third row, had survival rates of less than 70%, their low survival rates were attributed to water-logged condition in the planting areas.
- Eastern red cedar can assimilate ammonia better than the other five species. Therefore, considering only the foliar uptake, it will be the best option for controlling ammonia pollution from poultry buildings.
- In terms of the plants effectiveness in trapping particulate matter, results showed that Yaupon and Arborvitae effectively trapped significantly more particles with diameters of as much as 20 microns per leaf surface area. For particles between 5 and 10 microns, Arizona cypress, American holly, and Eastern red cedar trapped significantly more per surface area compared to the other three plant species.
- All six plant species can be used as bio-indicators since they were all classified as sensitive to pollution and will be useful for research studies.

INTRODUCTION

Project Justification

Texas ranked 6th in the nation in broiler production, producing 3.6 billion pounds and generating $1.8 billion in revenue in 2010. Texas broiler production has grown approximately 150% since 1990 (USDA-NASS, 2011). The continuing growth of poultry production in Texas, and intensive animal production systems in general, led to increased number of odor-related complaints from communities in close proximities to these facilities. Given the increased scrutiny from the public and policy makers, it is in the interest of poultry producers to look for control technologies that will abate the transport of odor and other emissions from their facility to the neighborhood.

Poultry houses generate significant amount of particulate matter (PM), ammonia, (NH_3) hydrogen sulfide (H_2S), and other odorous gases that are expelled to the outside environment through the exhaust fans in the buildings. These PM and gaseous emissions have been known to pose significant health and environmental concerns especially when the buildings are located close to residential, commercial, and recreational areas. Fine particulates (those with diameters under 2.5 μm), in particular, may pose the most health hazard since they can pass to lung alveoli when inhaled. In addition, particles may be vectors for microbes, toxic agents (such as metals and antibiotics), and odorous gases; when attached to fine particles, these pollutants can be transported in the atmosphere over long distances. Odorous gases trigger complaints from neighbors often resulting in lawsuits, moratoria on expansion of farms, and odor control regulations in some states. Although much current dissatisfaction is driven by concerns about odors, air quality concerns have also focused attention on nitrogen deposition and ammonia emissions for which poultry buildings and poultry manure application are sources.

A variety of strategies and control technologies are available for reducing emissions and dispersion of pollutants from animal structures. One control strategy that is natural and is receiving increasing attention is the use of vegetative shelterbelts (or vegetative environmental buffer) to capture farm emissions. Vegetative shelterbelts are rows of trees and other vegetation planted around poultry buildings or strategically located directly opposite of exhaust fans. Vegetation that are correctly placed will not only create visual barrier or provide shadings that could result in cooler microclimate during summer, but also provide filtration surfaces for PM and gases. When PM and gases are captured on plant surfaces, fewer pollutants will be picked up by the wind passing over the building. Consequently, the impact on the environment and human health and welfare is lessened by collecting and dispersing the pollutants before they reach the nearby community.

Gaseous pollutants such as NH_3 are removed primarily by uptake via leaf stomata while PM is removed from the pollutant stream by impaction and interception on plant surfaces. The removal rates depends on many factors such as the concentration and distribution of pollutants, meteorological factors (wind, precipitation), and plant size and species. Although the use of vegetation to enhance dispersion of pollutants and intercept dust is not a new concept, very limited studies that documented their effectiveness in controlling pollutants from poultry buildings have been reported in literature (e.g. Adrizal et al., 2008a; Malone et al., 2006; Patterson et al., 2008). The aforementioned studies suggested that vegetative shelterbelts

were effective in mitigating poultry odor and other emissions. However, there is a need to test the system's effectiveness using the native plants that are accessible to us in East Texas. Quantifying the ability of our native plant species to mitigate PM, NH_3, and other odorous gases will provide our poultry industry on-farm data that are needed to make informed decision on using vegetative shelterbelt as a Best Management Practice.

Project Description

This project utilized six different plant species (Arborvitae, Roughleaf dogwood, Eastern red cedar, Yaupon, American holly, and Arizona cypress) that were established in the zone directly opposite of the exhaust fans of four poultry buildings at SFASU's Broiler Research Center. The concentrations of PM, ammonia (NH_3), and odor were measured at 16 sampling locations in front of the exhaust fans and at the property lines. In year 1 of the project, baseline data were collected during the last week of two production cycles prior to shelterbelt establishment. In years 2 and 3, measurements were conducted during the last week of three production cycles. In addition to aerial concentration measurements, foliar concentration of ammonia and particulate loadings on leaves were also measured following the procedures in Appendices A and B, respectively. Field measurements were also supplemented with controlled laboratory experiment to test the effectiveness of the six plant species in absorbing ammonia as discussed in Appendix C.

Project Objectives

(1) Evaluate the effectiveness of various tree species in particulate matter and ammonia uptake, and in reducing odor;
(2) Assess the ability of trees to tolerate building emissions; and
(3) Examine management and costs requirements of using vegetative shelterbelts.

METHODS

Study Area and Shelterbelt Design

The study was carried out at SFASU's Broiler Research Center (BRC) that has four tunnel-ventilated broiler houses (Figure 1). Each house has 11 exhaust fans; six 52-in fans are located on one end wall, two 52-in fans each are on the adjoining sidewalls and one 36-in fan is on the other end wall. The exhaust fans were fitted with cones. Shelterbelt was established in the zone that was directly opposite of the 10 52-in exhaust fans. As seen in Figure 2, three of the plant species (Yaupon, American holly, and Arizona cypress) were planted in front of buildings 1 and 2 while the other three (Arborvitae, Roughleaf dogwood, and Eastern red cedar) were situated in front of buildings 3 and 4 (Figure 3).The buildings were populated with about 104,800 birds during the measurement periods.

Figure 1. The funnel-ventilated broiler houses at SFASU's Broiler Research Center. Building 1 is on the far right while building 4 is on the far left. Three rows of six plant species were planted directly in front of the exhaust fans.

Figure 2. Yaupon, American holly, and Arizona cypress were planted in front of poultry buildings 1 and 2.

Figure 3. Roughleaf dogwood, Eastern red cedar, and Arborvitae were planted in front of poultry buildings 3 and 4.

Measurement of Air Pollutants

Particulate matter ≤ 10 μm (PM_{10}) and ≤ 2.5 μm ($PM_{2.5}$) in diameters were measured using Haz-Dust samplers (Model EPAM 5000, Environmental Devices Corp., Plaistow, NH). Gastec passive dosimeter tubes and scentometers (Nasal Ranger, St. Croix Sensory, Lake Elmo, MN) were used to measure the concentrations of ammonia and odor, respectively. The NH_3 uptake of the plants was measured using the procedure discussed in Appendix A. Appendix B discusses the procedure for the determination of particulate loading. In addition to the field measurements, controlled laboratory experiment was also conducted to determine the ammonia foliar uptake by the plant species. The laboratory component was added because of the difficulty encountered in establishing four of the plant species in the field during the first year of planting. In the laboratory measurements, the foliar ammonia concentrations and physiological response (photosynthetic rate, stomatal conductance, and transpiration rate) of plants at varying levels of ammonia exposure were determined.

Plant Growth Analysis

For the plant growth analysis, the height and diameter at breast height were measured with a height pole and a caliper, respectively. The plants were also inspected at the beginning and end of every production cycle for the presence of pest infestation, wilting, and dieback. Difficulty in establishing the trees was encountered in the first year of planting resulting in replanting of Eastern red cedar, Roughleaf dogwood, Arborvitae, and Arizona cypress.

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Quality Assurance

All instruments that were used for sampling were calibrated either at the Environmental Assessment Lab or were sent back to the manufacturer for factory calibration. Data that were collected electronically from the Haz-Dust, weather station, pocket weather tracker, hobo dataloggers, and other instruments were saved to a flash drive and hard drives of the desktop computers in the Environmental Assessment Laboratory. Electronic and hard copies of all data were also be kept in Dr. Jerez's office computer and filing cabinet. Dr. Jerez was in charge of supervising the students during the calibration in the laboratory and data collection both in the laboratory and the field.

The effectiveness of various plant species on foliar ammonia uptake was determined with the two-way analysis of variance (ANOVA) procedure of SAS (version 9.2, SAS, Cary, NC). A repeated measure design with one fixed among subjects (species) and two fixed factors within subject (treatment levels, exposure) was used to determine significant differences on foliar ammonia content of plants. For particulate loading, significant differences were determined through one-way analysis of variance. Test of least significant differences was performed when the F-test was significant at 0.05 level. Data was pooled after a significant ANOVA with Tukey HSD procedure to compare the differences on the means.

FINDINGS

Evaluation of Effectiveness in Removing Particulate Matter, Ammonia, and Odor

Results of baseline measurements in year 1, prior to planting, showed that there was no detectable odor along the property line. Therefore, there was no further assessment done on the effectiveness of the plant species in odor removal. Furthermore, the ammonia concentrations measured at 10 sampling locations along the fence line showed concentrations ranging from less than 1 to 4 ppm for 18 days of sampling events in March 2013 and no measurable concentrations (< 1 ppm, which is the detectable limit) for five days of measurements in July 2013. For particulate matter less than or equal to 10 microns (PM_{10}) and less than or equal to 2.5 microns ($PM_{2.5}$) in diameter, the fence line downstream of the exhaust fans of buildings was located next to US 59, thus, attributing any PM_{10} and $PM_{2.5}$ concentrations to the emissions from the poultry buildings alone will be misleading. The difficulty encountered in establishing the seedlings in the field during the first year of planting resulting in several replanting events, combined with the barely detectable level of ammonia at the fence line, led to the revision of the original measurements plan. Instead of using the aerial measurements of particulate matter and ammonia to test the effectiveness of the plant species, the foliar ammonia concentrations and particulate loading on the leaves were measured using the procedures discussed in Appendices A and B. Fresh foliage samples of approximately the same size and at approximately the same height were collected randomly from the six plant species planted in the field. The fresh foliage samples were analyzed for ammonia concentration in the Environmental Assessment laboratory at SFASU. Field measurements were also supplemented with controlled laboratory experiment to test the effectiveness of the six plant species in absorbing ammonia using the methodology discussed in Appendix C.   In the analysis of the foliar ammonia concentrations of leaves collected from the field, a split-plot design resulted in

Eastern red cedar with the highest foliar ammonia concentration followed by American cypress, Arborvitae, American holly, Roughleaf dogwood, and Yaupon (Figure 4). This result was further confirmed in the controlled experiment in the laboratory.

In the controlled study in the laboratory (Appendix C), seedlings of the six plant species were exposed to three treatment levels of ammonia (1, 5, and 10 ppm) for 1 hour each time and leaves were collected afterwards for the foliar ammonia quantification following the procedure discussed in Appendix C. Results showed a significant difference in foliar ammonia concentrations among the six plant species. Eastern red cedar had the highest foliar ammonia content while Yaupon had the lowest as seen in Figure 5. In other studies (e.g. Adrizal et al., 2008b; Adriaenssens et al., 2010), deciduous trees were found to incorporate more ammonia into their tissue and plant foliage compared with evergreens. In this study, however, Eastern red cedar, which is an evergreen, yielded the highest foliar ammonia content. According to Adrizal et al. (2008b), the leaf surface area can be a factor for the higher efficiency of conifers to absorb ammonia. Adriaenssens et al. (2010) related this higher efficiency to lower nitrogen demand of conifers because of lengthy needle retention and efficient internal nitrogen recycling. For plants measured with lower foliar ammonia content (e.g. Yaupon, Arborvitae), assimilation rate might have been limited due to compensation capacity of plants. As can be seen in Figure 6, regardless of concentration levels of ammonia exposure, all six plant species had higher foliar ammonia concentrations compared to the control indicating that all six species were able to assimilate ammonia at varying effectiveness.
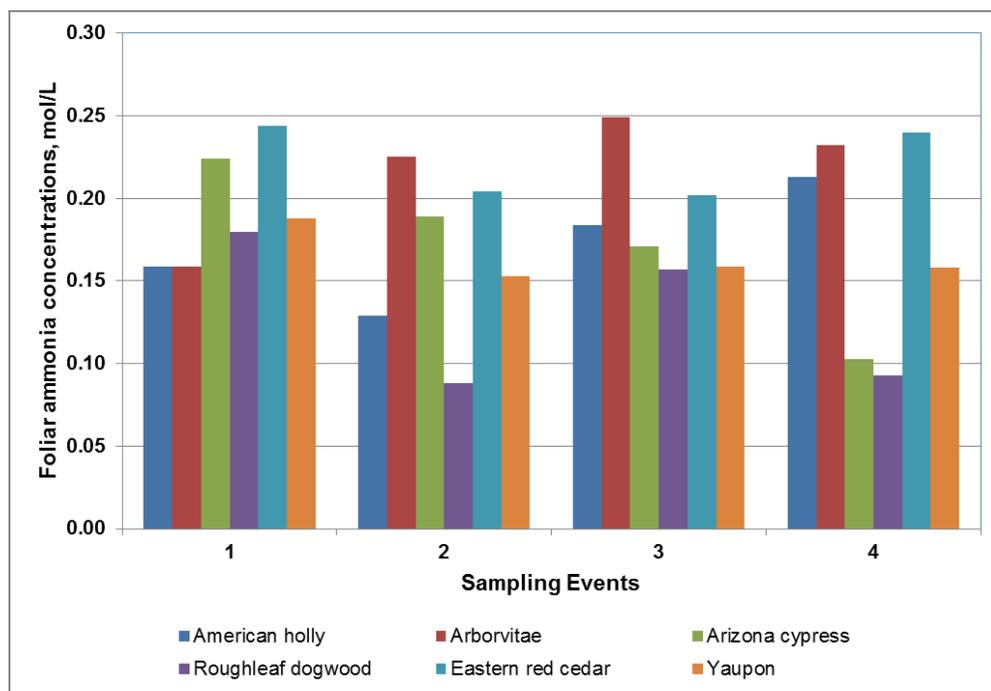
Figure 4. Comparison of means of foliar NH_3 content of the six plant species planted in the field.
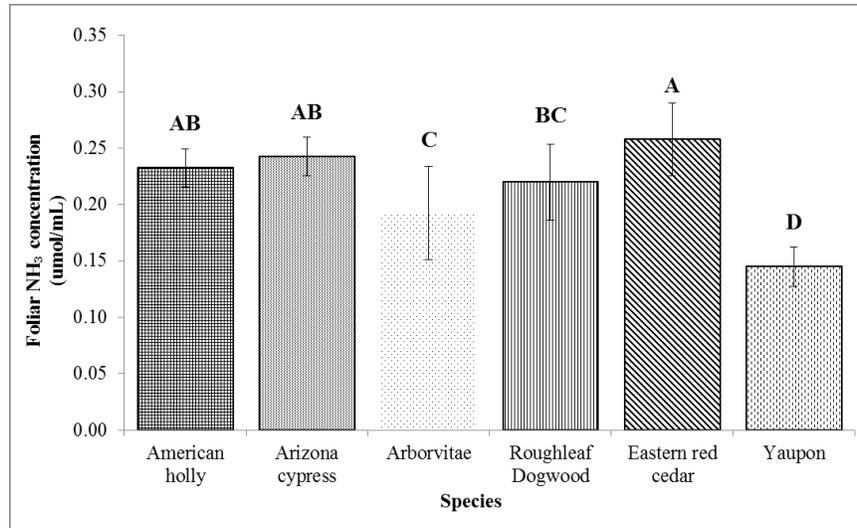
Figure 5. Comparison of means of foliar NH$_3$ content of the six plant species among all treatment levels. Error bars represent standard deviation. Groups with the same letter are not statistically different at the 5% level.
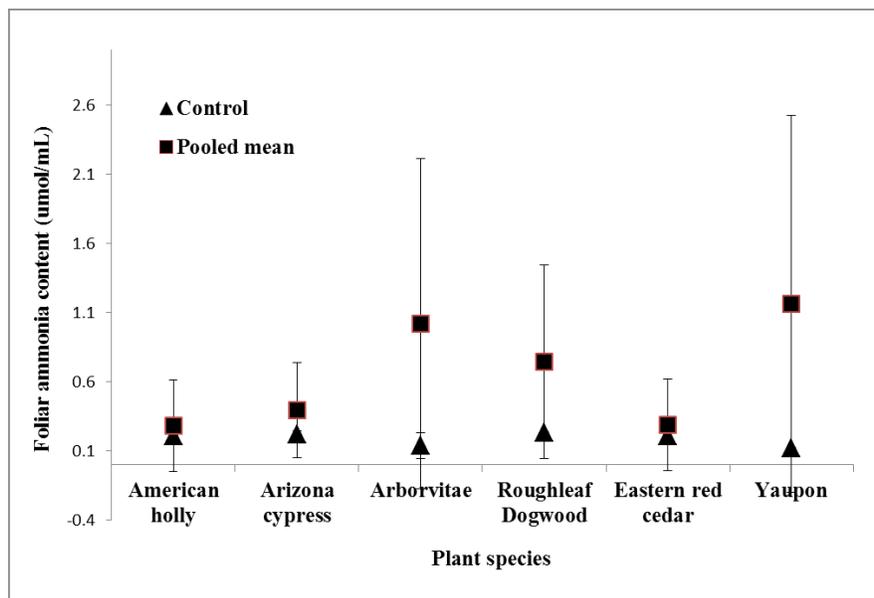
Figure 6. Pooled foliar ammonia content at three treatment levels in comparison to control plants. Error bars represent standard deviation.

In addition to the measurement of foliar ammonia concentrations, the air pollution tolerance index (APTI) and physiological responses (i.e. net photosynthetic rate, stomatal conductance, and transpiration rate) of the six plant species were also determined in the laboratory. The APTI

is a function of the ascorbic acid, total chlorophyll, and pH, and relative water contents and was calculated using the equation below.

$$APTI = \frac{[A(T + P) + R]}{10}$$

where A = ascorbic acid content, T = total chlorophyll content, P = leaf extract pH, and R = relative water content. An APTI value ranges from 0 to 100 with <1 considered as very sensitive, 1 to 16 as sensitive, 17 to 29 as intermediate in sensitivity to pollution, and 30 to 100 as being pollution tolerant (Agbaire and Esiefarienrhe, 2009). The effect of exposure to ammonia on the physiological responses of plants was determined using a photosynthetic gas exchange system (Model 6400, Li-Cor Instruments, Lincoln, ME) as discussed in Appendix C. The APTI and physiological responses are considered as good indicators of the plants tolerance to pollution. As discussed in Appendix C, exposure to ammonia significantly lowered the photosynthetic activity of Eastern red cedar, Arizona cypress, and American holly. Although lowered, the values were still within the range expected for conifers and evergreen plants which means that their photosynthetic activities will not be limited. In addition, ammonia exposure caused increased stomatal conductance and transpiration rates on American holly and Arizona cypress while Eastern red cedar responded differently. The results of APTI measurements showed that all six plant species were classified as sensitive to pollution and will be useful as bio-indicators in pollution studies.

The potential of foliage of different plant species to trap particulate matter was also evaluated to determine their effectiveness in controlling pollution. The procedure and results of this study was presented in detail in Appendix B.

Assessment of Tolerance to Building Emissions
In the assessment of the trees/shrubs tolerance to emissions, only those planted in three rows directly in front of the six bank o exhaust fans were used. The trees/shrubs used as controls and those planted in front of the sidewalls were excluded since they were either not directly exposed to pollution or were not adequately maintained so factors other than emissions could have affected their performance. Shown in Table 1 is the comparison of their survival rate determined between the last planting event in April 2014 and September 2016. Yaupon and Roughleaf dogwood were both planted in the first row and also had the highest survival rate (96 and 92%, respectively). Arborvitae, which was planted in the third row in front of buildings 3 and 4, had the lowest survival rate of 32%. The low survival rate for Arborvitae can be attributed to the water-logged condition in front of building 4, where no Arborvitae survived after three replanting. Shown in Figure 7 is the row of Arborvitae that survived in front of building 3.

Table 1. Comparison of percent survival rates of the six species of trees/shrubs planted directly in front of the four poultry buildings.

Trees/shrubs	Original #	# dead	% survival
Yaupon	24	1	96
American holly	24	4	83
Arizona cypress	24	9	63
Roughleaf dogwood	24	2	92
Eastern red cedar	25	3	88
Arborvitae	25	17	32

Figure 7. Only the Arborvitae planted in the third row in front of poultry building # 3 survived. Those planted in front of building # 4 were in water-logged condition.

Ocular inspection of leaves were done during the sampling events. The following are some of the observations on visual injury on the plant foliage (Figure 8):

- Yellowing/brown discoloration of *Arborvitae* needles pronounced on tips;
- Red/brown spots are numerous and brown discoloration are distinct on the edges of *Roughleaf dogwood* leaves;
- Waxy leaves of *American Holly* have traces of black spots which might be an early sign of necrosis;
- Dark discoloration effect was observed on the leaf edge of Yaupon leaves;
- *Eastern red cedar* seems to effectively trap and collect dust particles through their needle-like foliage; and
- No signs of visible injury or change on leaf colors observed on *Arizona cypress* and *Eastern red cedar*.

Figure 8. Visual injury observed on some leaf samples.

Economic Analysis

Eastern red cedar, Roughleaf dogwood, Arborvitae, and Arizona cypress were replanted three times during the first two years. In the following economic analysis, only the trees planted last and were directly in front of the six bank of exhaust fans were used in the calculations. The trees that were used as controls and those planted in front of the sidewall fans were excluded since they were either not directly exposed to pollution or were not adequately maintained because of the difficulty accessing their locations. Fixed costs include the cost of procuring the seedlings, installing the irrigation system, and building a fence. Labor for planting included five student workers paid at $8/hr for 40 hours total per worker. Installation of the irrigation system and building the fence required three student workers working for about 24 hours each. Variable costs included mowing every week from spring to fall and once a month during winter. Irrigation was for 270 days with each tree receiving about 1 gallon of water per day at a cost of $0.004 per gallon. With fixed costs spread over 10 years, the total costs of the system were $1776.64/year. The buildings housed an average of 104,800 birds per flock; with five flocks per year, the estimated additional cost of the system was $0.003/bird.

Table 2. Economic analysis on the cost of establishing and maintaining a shelterbelt.

Cost Items		Materials ($)	Labor ($)	Total Cost ($)
Fixed Cost	Yaupon	288.00		
(10 years)	American holly	768.00		
	Arizona cypress	456.00	1,600.00	5,188.00
	Roughleaf dogwood	552.00		
	Eastern red cedar	1,125.00		
	Arborvitae	399.00		
	Irrigation system	800.00	576.00	3,276.00
	Fence	1,900.00		
			Total Fixed Cost	**8,464.00**
Fixed cost per year				
spread over 10 years				**846.40**
Variable Cost				
	Mowing			
	(36 hours per year; 1 hour each)			720.00
	Irrigation			
	(1 gal/tree; 270 days a year; $0.004 per gallon)			157.68
			Variable Cost/yr	**877.68**
			Total cost/yr	$ 1,724.08
			Total cost/bird	$ 0.003

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CONCLUSIONS

- All six plant species exhibited tolerance to poultry building emissions. Yaupon and Roughleaf dogwood that were planted in the first row and received the most amount of pollution had survival rates of over 90%. Although Arborvitae and Arizona cypress, which were planted in the third row, had survival rates of less than 70%, their low survival rates were attributed to water-logged condition in the planting areas.
- Eastern red cedar can assimilate ammonia better than the other five species. Therefore, considering only the foliar uptake, it will be the best option for controlling ammonia pollution from poultry buildings.
- In terms of the plants effectiveness in trapping particulate matter, results showed that Yaupon and Arborvitae effectively trapped significantly more particles with diameters of as much as 20 microns per leaf surface area. For particles between 5 and 10 microns, Arizona cypress, American holly, and Eastern red cedar trapped significantly more per surface area compared to the other three plant species.
- All six plant species can be used as bio-indicators since they were all classified as sensitive to pollution and will be useful for research studies.

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APPENDICES

Appendix A – Draft of Paper: Atmospheric Ammonia Content of Selected Plant Species in a Controlled Chamber

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Abstract

Uptake of atmospheric NH_3 was investigated using six plant species comprised of Yaupon, Eastern red cedar, American holly, Arizona cypress, Arborvitae and Roughleaf dogwood in a controlled-chamber at three treatment levels corresponding to increasing NH_3 (1, 5, 10 ppm) concentrations. Uptake rate as measured through NH_3 concentration values were not found to be statistically different (p=0.12) among all the species after exposure in the controlled chamber. Using a modified method on enzymatic analysis, foliar ammonia content (μmol/mL) of Eastern red cedar plants exposed to NH_3 was significantly high than non-exposed plants to ammonia (p=0.0003).

Keywords: controlled-chamber, enzymatic technique, foliar ammonia content, plants

Introduction

Anhydrous ammonia ("without water") is a colorless gas (NH_3) with a very sharp odor and exists naturally at levels between 1 and 5 parts in a billion parts of air (ppb). As manure and dead organisms decompose, NH_3 returns to the soil and atmosphere then comprised most of NH_3 naturally found in the environment. On one hand, fertilizers comprised eighty percent of all manufactured NH_3. Ammonia only has a short span of time to exists in the environment as it is recycled naturally, incorporated and transformed by plants and microorganisms (ATSDR, 2004).

Atmospheric NH_3 enters the leaves of higher plants via the stomata and is dissolved in the water film of the mesophyll cells to form NH_4^+. Ammonium is the reduced N form used by plants for assimilation into amino acids and proteins. The ammonia stomatal compensation points of plants were also determined by leaf temperature, NH_4^+ concentration and pH of the apoplastic solution (Massad et. al., 2008).

The current knowledge on NH_y (NH_3 & NH_4^+) effects on plants was based on studies using different approaches including experimental designs using controlled exposures or direct field studies in which physical climate and pollution climate may not be controlled (Fangmeier et. al., 1994). Climate conditions and plant physiological functions both aid the exchange of NH_3 between plant system and the atmosphere. Effect of management factors in agriculture also specifically influenced soil nitrogen parameters (Mattsson et.al., 2009). Use of common oak leaf (Quercus robur L.) characteristics in passive monitoring of ambient NH_3 concentration didn't show relationship on its effect and changes on morphology, anatomy and physiology of plants located closer to livestock farms (Wuytack et. al., 2013). Measurements in the field however pose environmental and meteorological conditions that relatively vary affecting response of plant's uptake of NH_3.

Plant's (e.g. soybean, corn, cotton and soybean) absorption of NH_3 was monitored through an airstream flow in a small growth chamber and NH_3 were observed to lower concentration values (Hutchinson et.al., 1972). In Sutton et. al. (1995) study, a controlled environment provided useful information in determining plant-atmosphere exchange of NH_3 as it demonstrated existence of a compensation point concentration for NH_3 transport within plant stomata to the atmosphere. Van Hove (1990) observed wind velocity; air temperature and vapor pressure deficit of the air (VPD) influenced plant's absorption of NH_3 on bean leaves in an experiment using a leaf chamber. Aneja et. al. (1986) supported dry deposition of NH_3 at environmental concentrations of six plant species through their deposition velocity.

Leaves of cotton and corn were dried and analyzed for carbohydrate content and nitrogen fractions. It is necessary for the plant material to be dried up for the purposes of facilitating sampling and reducing risk of degradation and other chemical changes during storage (Marur & Sudek, 1995). Navel oranges fresh and oven-dried leaves were analyzed for ammonia using various method of preparation, processing, extraction and storage method. Oven-dried leaf samples were compared and detected to have greater NH_y concentrations than aliquots of the same leaves analyzed after freeze drying and analysis immediately after

collection. Stored ground-oven-dried orange leaf tissue in 10% TCA at -20ºC for one week did not affect NH_y concentration of the samples. Stored filtered homogenate of ground-oven-dried orange leaf tissue in 10% TCA at 4 or -20ºC resulted in a slight (12%) increase in leaf NH_y concentration of the samples (Ali & Lovatt, 1995).

The overall objective of the experiment was to design and assess the performance of a static closed exposure chamber for measuring gaseous uptake by plants for short time duration. As previously mentioned, while most early physiological studies on field is deemed important, certain reasons and possibilities arise that studies in the laboratory can also be useful and sometimes help to validate and gather supplemental data. To further support uptake of NH_3, a modified method in quantifying the amount of ammonia absorbed by the leaves was developed and tested based on exposure chamber measurements.

Materials and Methods

Experimental Set-up. A controlled-chamber was designed and constructed to ensure containment of NH_3 and prevent escape of the gas. The chamber (81.80 Liter capacity) was made up of Plexiglas material (40.64 cm x 30.48 cm x 66.04 cm) and lined up with polyethylene foam tape (3M Double Coated Tape, St. Paul, MN 55144) together with a laboratory film (Parafilm "M", Chicago, IL 60631) inside to prevent chemical reaction with the airflow. Several inlets were drilled into the chamber for flexibility; two openings in the chamber served as the inlet and pathway for NH_3 injection point and an outlet for an NH_3 detector tube to determine the concentration inside. All inlets were covered with rubber stoppers and supplemented with Parafilm tape. Inner walls and sensors that were exposed inside the chamber were coated with Teflon material (Dry-Film Lubricant, Du Pont, Bay Shore, NY) to avoid ammonia reaction with the wall surfaces of the chamber. Air circulation in the chamber was facilitated by an axial fan (Axial 1225, Muffn 115V AC Cooling Fan, City of Industry, CA) mounted through a 1" stainless steel rod attached inside the chamber. The fan was kept in continuous operation to reduce space variability of gas concentration and reduce the boundary resistance layer of the leaf (Van Hove et.al., 1987). The air velocity was monitored using a hot-wire anemometer (Model 9545-A, TSI Inc., Shoreview, MN). Temperature, relative humidity and light intensity were also monitored throughout the experiment using Hobo Data loggers (HOBO U-12 Logger, Onset Computer Corporation, Bourne, MA). The chamber was tested for leak using the Nextteq Irritant Smoke Tube Kit (P/N 9501 irrita, Nextteq LLC, Tampa, FL) to check escape of gas from the chamber. It took approximately one minute for the smoke to cover the entire chamber. The axial fan was turned on and allowed to run for about 15 minutes. Visual inspections did not indicate any leak.

Sampling Duration. Three ammonia concentrations served as treatment levels covering 1 ppm, 5 ppm and 10 ppm. Injection of NH_3 started as soon as the chamber was sealed and secured air-tight at a flow rate of 0.5 liters per minute (lpm) for one hour. Ammonia was supplied to the chamber as a compressed gas from a 1R (29.50 Liters - internal volume) cylinder size (Matheson Tri-Gas Company) using polytetrafluoroethylene tubing and stainless steel material. The delivery of gas into the chamber was controlled by a stainless steel regulator and metered by a glass tube flowmeter (Series GS, Key Instruments, Hatfield, PA). Ammonia concentration inside the chamber was measured using the NH_3 passive dosimeter tubes (Ammonia 2/a, Drager Safety, Atlanta, GA) prior to every start of a new exposure and after the designated sampling duration.

Exposure to Ammonia. Six plant species comprised of Yaupon (Ilex vomitoria), Eastern red cedar (Juniperus virginiana), American holly (Ilex opaca), Arizona cypress, Arborvitae (Thuja plicata x standishii) and Roughleaf dogwood (Cornus drumondii) were selected as test species for measurement on the uptake of ammonia in the chamber. Plants were obtained from

commercial sources and transferred to individual one-gallon polypropylene pots with a planting medium (Hyponex by Scotts ©Potting Soil). Plant heights ranged from 20 to 50 cm and at around a year to two years old seedlings and kept in the greenhouse for two months for stabilization prior to exposure.

Plants were watered to field capacity to ensure that plant's physiological responses were not due to water deficiency that eventually affects plant-water relationship. Soil volumetric water content (VWC) was measured using a soil moisture meter (Field Scout TDR 100/200, Spectrum Technologies, Plainfield, IL) and ideally VWC values were maintained above 10%. Parafilm sheets were used as plant pots cover to avoid NH_4^+ assimilation through the roots.

Leaf samples from Eastern red cedar plants were randomly collected from exposed plants for foliar ammonia content using a modified method of enzymatic technique (Sicher & Bunce, 2008). Eastern red cedar was chosen as test species in the preliminary experiment after results showed lowest NH_3 concentration measured in chamber at all treatment levels as compared to other five species.t. It was ideally used as a buffer strips around parking lots, median strip plantings in highway and as a reclamation plant. It successfully grows in urban areas where air pollution, poor drainage, compacted soil, and/or drought were common (U.S. Forest Service, 1993). All results from enzymatic technique were expressed as micro moles per milliliter of leaf extract (umol/mL).

Ammonia Quantification. An established procedure on enzymatic analysis by Kun & Kearney (1974) was utilized to analyze foliar NH_3 which was modified to Ali & Lovatt (1995) procedure to suit the type of leaf samples for analysis (sample preparation, extraction, storage and recovery of combined NH_3 and NH_4^+. In this analysis, dried-leaf samples were used instead of fresh leaf samples to ensure a clean, free of extraneous substances (e.g. soil, dust) leaf samples that can influence analytical results (Plank, 1992) since all test species have been kept in the greenhouse for three months prior to the experiment and exposed to other plants as well. The enzymatic method by Kun & Kearney (1974) was modified further in terms of spectrophotometric calibration results involving the volume of distilled water, plants extract and NADH used. An original volume of 150 μL of DW was changed to 2000 μL; 500 μL of plant extracts to 100 μL; and 30 μL to 200 μL of NADH. These changes on final volume were made after a sound calibration and linear relationship was attained between NH_3 standard concentrations and their corresponding absorbance on the instrument.

Leaf samples were washed first with 0.2% detergent solution and 0.1M HCl to remove greasy coating on leaf surface and eliminate NH3 in the leaf during dry deposition. Then, leaf samples were prepared prior to extraction by drying method. Instead of using fresh weight samples, dry powdered samples were utilized for extraction of NH3 content. It has been suggested (Marur & Sudek, 1995) that when dried plant materials are subjected for plant analysis, chemical changes during storage are lessened. When leaves were brittle enough, reducing them into powder form was immediately performed. When storing large amount of leaf samples, it is necessary that certain temperature conditions are met as NH_3 can escape and volatilize under room conditions. During storage, plant samples were kept at -20ºC to avoid degradation of samples. Oven-dried leaf samples (200 mg) were then added to a centrifuge tube with 2 mL of Trichloroacetic acid (TCAA,10% w/v). Trichloroacetic acid served as an extracting solution of NH3 in the plant extracts. To ensure homogenization, leaf samples underwent centrifugation process. Extracts were then neutralized and prepared for enzymatic treatment. As plant extracts were treated with enzymes, co-enzymes and buffer, extinction started immediately and stopped at a certain period when all NH_3/NH_4 ions were used up during the entire reaction process.

Statistical Analysis

A complete balanced randomized design was used to assess the performance of a static closed exposure chamber for measuring NH_3 uptake by plants (ppm). Significant difference on NH_3 chamber concentration and foliar uptake of NH_3 were determined with two-way analysis of variance procedure (species$_{factor1}$ & treatment levels$_{factor2}$). Three levels of treatment (Low, Medium and High) on the six plant species was replicated three times and significance were determined through the calculated F-value. The measurement of foliar uptake of NH_3 (umol/mL) was replicated three times (N=108, n=18).Significant difference on foliar NH_3 content of Eastern red cedar needles was analyzed with one-way analysis of variance procedure (N=24, n=6). All statistical analysis was performed using SAS statistical software (Version 9.2, SAS, Cary, NC).

Results and Discussion

Design and assessment of the performance of a static closed exposure chamber for measuring gaseous uptake by plants

Plants were exposed at three defined concentrations of ammonia into a controlled-chamber for one hour. Concentration values was immediately measured and were found to be not statistically different (p=0.12) among all the species after exposure in the controlled chamber. However for treatment levels, the high level of treatment (10ppm/hour) was found to be statistically high (p<0.0001) than low and medium treatment (Table 1). Ammonia concentrations were 0.65 ppm (American holly & Eastern red cedar plants), 0.78 ppm (Arizona cypress), 1.53 ppm (Roughleaf dogwood), 2.33 ppm (Arborvitae) and 2.67 ppm (Yaupon). Low level treatment barely made a measurable amount detected as observed on Arizona cypress (0.12 ppm), Roughleaf dogwood (0.18 ppm) and Yaupon plants (0.02 ppm). Detector tube was unable to measure anything (0 ppm) during exposure treatment for American holly, Arborvitae and Eastern red cedar plants. Readings during medium level of treatment were observed to be lowest from American holly and Eastern red cedar (0.22 ppm), followed by Arizona cypress (0.28 ppm), Roughleaf dogwood and Arborvitae (0.53 ppm) and highest in Yaupon plants (0.8 ppm).

Table 1. Ammonia concentrations (mean values + standard deviation) measured in the controlled chamber when occupied with the tested plant species. The injected NH3 standard concentrations of low, medium, and high corresponded to 1, 5, and 10 ppm. Significant difference at p< 0.05.

Exposure levels	NH3 concentration in the controlled chamber (ppm)		
	Low	Medium	High
American holly	0 ± 0	0.2 ±0.26	0.65± 0.52
Arizona cypress	0.12 ±0.16	0.28± 0.19	0.78± 0.20
Arborvitae	0 ±0	0.73± 0.46	2.33± 1.53
Roughleaf Dogwood	0.18± 0.28	0.53 ± 0.05	1.53± 0.20
Eastern red cedar	0± 0	0.22± 0.08	0.65± 0.38
Yaupon	0.02± 0.03	0.80± 1.04	2.67± 2.89

Modified method on enzymatic analysis to quantify foliar NH$_3$ content

To test sampling duration's substantial change on the foliar ammonia concentration, Eastern red cedar needles were taken from the exposed plant and analyzed for foliar ammonia content in the laboratory in comparison to non-exposed plants (Table 2). Mean foliar NH$_3$ content of non-exposed plants were significantly lower than gradual increasing NH$_3$ concentration of plants exposed at three treatment levels (0.21 umol/mL vs 0.28 umol/mL). Figure 1 clearly shows the trend of foliar NH$_3$ content of Eastern red cedar plant species measured through enzymatic analysis to injected NH$_3$ standards in the controlled-chamber.

Table 2. Foliar ammonia concentration in Eastern red cedar needles after 1-hour exposure to ammonia at different treatment levels.

Species Number	NH$_3$ Concentration of plant samples (umol/mL)			
	Control	Low	Medium	High
Spp1	0.24	0.26	0.26	0.27
Spp2	0.25	0.26	0.26	0.27
Spp3	0.26	0.28	0.26	0.27
Spp4	0.18	0.27	0.28	0.27
Spp5	0.18	0.27	0.28	0.28
Spp6	0.17	0.27	0.27	0.31
Mean	0.21	0.27	0.27	0.28
S.d.	0.04	0.01	0.01	0.02
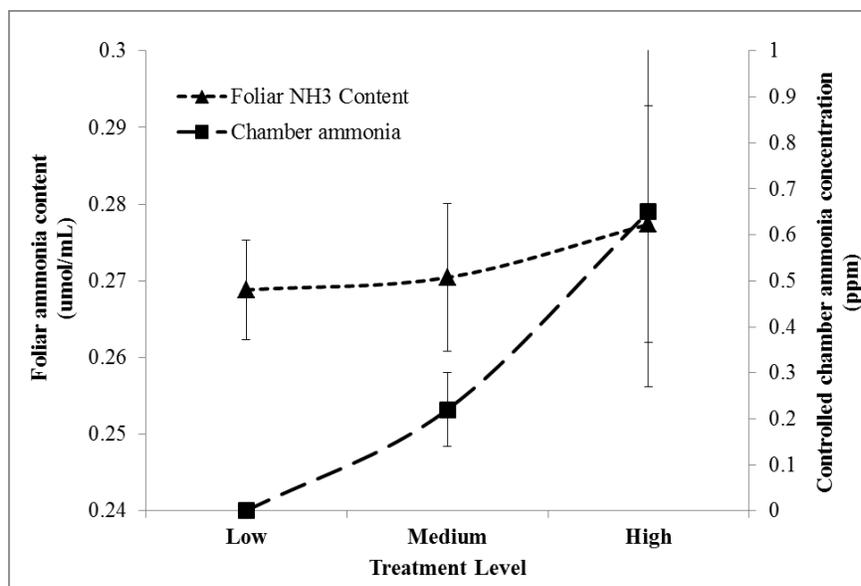
Figure 1. Ammonia concentration (ppm) in controlled-chamber at three treatment levels and foliar ammonia content (umol/mL) of Eastern red cedar plant species.

Results showed a significant statistical difference on foliar ammonia content between exposed and non-exposed leaves of Eastern red cedar plants (p=0.0003) but not for every treatment levels (Figure 2).
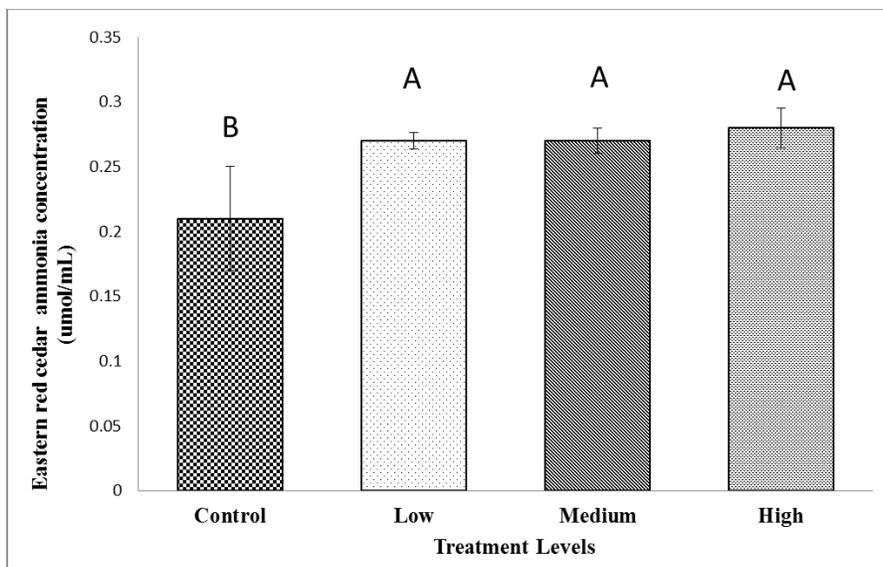
Figure 2. Foliar ammonia content (umol/mL) of Eastern red cedar plant species at four treatment levels. Treatment level with the same group is not statistically different. Error bars represent standard deviation.

Conclusion

The designed controlled-chamber was effective for measuring NH_3 uptake by plants. In future study on measuring the gaseous uptake by plants, the same design of a static exposure chamber was effective can be used if short-term exposure is intended. However, wall effect losses due to NH_3 adsorption to the controlled-chamber surface should also be considered in future experiments. The ability of plants to in atmospheric NH_3 uptake varies among plants but both American holly and Eastern red cedar tend to absorb more NH_3 on this study. The modified method of enzymatic technique successfully quantified foliar NH_3 content and showed significant difference between exposed and non-exposed Eastern red cedar plants.

Acknowledgement

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Appendix B – Draft of Paper: Particulate Matter Capture of Plants Used as Shelterbelts around a Poultry Farm

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Abstract

Plant species comprised of American holly (*Ilex opaca*), Arborvitae (*Thuja plicata x standishii*), Arizona cypress (*Cupressus arizonica*), Eastern red cedar (*Juniperus virginiana*) and Yaupon *(Ilex vomitoria)* established as shelterbelts around a poultry farm were studied on their efficiency on particulate matter capture and its effect on plant's stomata. Particulate matter adsorbed on leaf surfaces were quantified through gravimetric analysis and were found significantly different among species at particulate matter sizes of PM_{20} (p=0.0001) and $PM_{2.5}$ (p=0.0002). Using stomatal imprints and leaf discs, particulate matter trapped into intercellular parts (e.g. leaf cuticle) of the leaf were inspected and categorized into four particle sizes. Only particulate matter size division of $PM_{<2.5}$ (p=0.0211) and $PM_{>5, <10}$ (p=0.0173) were found to be significantly different upon deposition on plants. Stomatal characteristics of Arizona cypress plants were significantly (p<0.0001) affected compared to other plants. Stomatal length (p=0.3969) and stomatal pore size (p=0.1187) were not observed to be significantly different among all species.

Keywords

Particulate matter loading, intercellular deposition, gravimetric analysis, stomata

Introduction

Plants play an important role in combating air pollution and working as a 'natural pollutant sink' (Singh & Sinha, 2010) especially for the exposed surface of trees (e.g. bark, leaves) as they provide sites for the gravity or wind-blown settlement of particulates (Matyssek, et. al., 1995). Plants reduce air pollution by intercepting suspended particulate matters (SPM) and aerosols and retain them on the leaf surface (Singh & Sinha, 2010). Leaves are the primary route of the uptake which is controlled by the stomatal aperture and conductance to gas diffusion (Mansfield & Pearson, 1996). Vegetation constantly exposed to the atmospheric pollutants may absorb, accumulate and integrate pollutants impinging on the foliar surfaces (Radhapriya, 2012) but only as a temporary retention site for many atmospheric particles (Nowak, 2002).

Particulate matter refers to the mixture of solid and liquid particles suspended in the air which will form an aerosol that vary in sizes, shape, surface area, chemical composition, solubility and origin (Bidlak & Jansky, 2011) categorized in ambient air as tri-modal (Pope & Dockery, 2006) ranging from ultrafine particles to fine particles to coarse particles present in the atmosphere. Coarse particles are derived primarily from suspension or re-suspension of dust, soil or other crustal materials from roads, farming, mining, windstorms, volcanos and other natural processes which may be in the form of sea salts, pollen, mold, spores, and other plant parts. Fine particles are derived primarily from direct emissions from combustion processes which may consist of transformation products such as sulfate and nitrate particles. Ultrafine particles may also result from vehicle exhaust and atmospheric photochemical reactions that coagulates to form larger complex aggregate or may translocate from the lung to the blood and other parts of the body. On conventional particle deposition theory, larger particle sizes with diameter greater than 10 μm effectively show efficient rates of gravitational settling and deposition onto vegetation (Chamberlain, 1975). Another study shows particles larger than 5 μm has interception and impaction increasingly efficient while particles in the size range of 0.1 μm to 5 μm becomes inefficient in transport and capture of particles on vegetation. Particles smaller than 0.1 μm effectively transport through the viscous sub-layer by Brownian diffusion providing significant deposition rates (Fowler et al., 1998).

In confinement poultry houses, dust is one of the primary contaminant of concerns at present average airborne concentrations (Adrizal et al, 2008). Broiler-rearing facilities with over 100, 000 birds in South Norfolk, United Kingdom become one significant source of fine

particulate matter (PM$_{2.5}$) in substantial emissions of 6 tons per year in the area (Bull, 2007). Adrizal et. al., (2008) mentioned that TSP and respirable dust concentrations of 4.4 mg/m^3 and 0.24 mg/m^3, respectively, in confinement poultry houses may pose adverse environmental and health impacts to nearby communities. Establishment of plant shelterbelts along poultry buildings can serve as buffer from neighborhood. Planting trees around livestock buildings helps minimize the dispersion of dust to the environment (Malone & Wicklen, 2002) but might impact plant shelterbelts survival. Particulate deposition on the leaf surface shows cuticle injury and increase in the epidermal cell and stomata size and frequency on the plants as the main receptor continuously exposed to roadside air (Kulshreshtha et al, 2009).

Gostin (2009) found out that plants growing on industrial areas and near the major roads were observed to have significant decrease in the size of the stomata and increase on stomatal density of the leaves. Dust deposition on leaf cuticle due to particulate penetration into the epicuticular wax may reduce light incidence and reduce net photosynthesis (Singh & Sinha, 2010). In metropolitan areas, smoke particles that settle out of the air and accumulate as film on plant surfaces, cutting down the amount of light on plants. Foliage with sticky or hairy surfaces suffers the most (Daubenmire, 1974). Rice plants (*Oryza sativa*) , situated one kilometer away from a cement factory, were shown to receive high dust loads and observed to have lower biomass by 44% to 60% (Singh et al., 1990). Plant's capacity to hold dust or particulate matter (PM) can be species-dependent. Trees take up more pollutants, including PM, than shorter vegetation (Singh & Sinha, 2010). Malone (2004) reported reduction of dust by 50 to 53% at a distance of 14.6 meters downwind of a roaster house beyond three rows of trees of cypress and red cedar but does not specifically describe PM fractions trapped by different plant species.

This study examined the capacity of several species of trees on intercepting particulate matter from poultry buildings. The potential of foliage of different species to trap particulates was evaluated to determine the effectiveness of plants in controlling air pollutant dispersion. Furthermore, stomatal features were assessed to determine possible of particulate matter deposition on the leaf surface and into the leaf cuticle.

Materials and Methods

Field site and Species selection

Plants used as shelterbelts around the vicinity of the SFASU Broiler Center of SFASU's Water C. Todd Agricultural Research Center in Nacogdoches, Texas were selected for the experiment. Five species of plants which were a mixture of shrubs and conifers, moderately to fast growing trees were used as test species. These were American holly (*Ilex opaca*), Arborvitae (*Thuja plicata x standishii*), Arizona cypress (*Cupressus arizonica*), Eastern red cedar (*Juniperus virginiana*) and Yaupon (*Ilex vomitoria*).

Laboratory Measurement

Twenty (20) leaf/fascicles samples consisted of the most matured leaves were selected and taken at random points from low to mid-crown and more exposed branches. Fresh plant foliage was collected from each plant species and brought to Environmental Assessment Laboratory at Stephen F. Austin State University for particulate matter loading, particle counting and stomatal observations analysis (Figure 1).

Particulate matter loading

Particulate matter loading was analyzed through particle gravimetric analysis. A 0.02% solution of heptamethyltrisiloxane (>98.0%, Tokyo Chemical Industry, Portland, OR) was used as a surfactant (Adrizal et.al., 2007) to completely remove the particulate matter from each filter. Foliage samples were transferred to a flask after rinsing the collection bottles with distilled water

which was also added to the flask. Distilled water (125 mL) was added to each flask containing 95 µL of heptamethyltrisiloxane to prepare 0.02% surfactant solution. Flasks were stoppered and placed in the refrigerator and soak for 24 hours. Flasks were placed on a reciprocating shaker (Eberbach Corp, Arbor, MI) at 200 rpm and kept in operation for 30 minutes. Leaf samples were sprayed vigorously on all sides and removed from the flask allowing the distilled water to be collected in the flask. Solution was then successively filtered using three different pore sizes of filter papers suited for gravimetric analysis. The weight of particulate matter were subjected to gravimetric analysis using 55-mm diameter hardened-ash less filter paper with three different pore diameter to separate particulate matter sizes of $PM_{2.5}$ (Whatman 42), PM_8 (Whatman 40) and $PM_{>25}$ (Whatman 541). A digital microbalance (AB104-S Line Balance, Mettler Toledo LLC, Columbus, OH) was used to measure the initial and final weight of the filter paper after overnight drying of filter paper in the desiccator (Dry Keeper, C-Type, Frederick, MD).

Mean leaf area was derived from five scanning operations and used as measured value for leaf area (cm^2) using a leaf area meter (Model CI-202, CID Bio-Science Inc., Camas, WA). Particulate loading calculation was measured based on weight of dust on pre-weighed and final weight of filter paper per foliage area (g/cm^2) as shown in Equation 1.

$$W = \frac{W^2 - W^1}{A}$$

Where:

W = dust loading (g/cm^2)
W^2 = final weight (g)
W^1 = initial weight (g)
A = total leaf area, cm^2
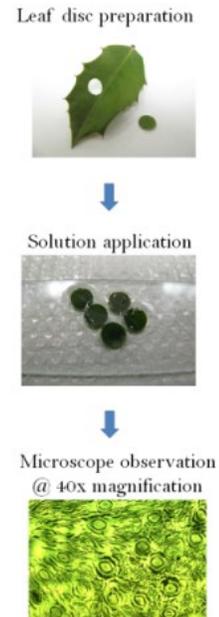
Figure 1. Laboratory procedures on particulate matter loading, particle counting and stomatal observations analysis.

Leaf particulate matter deposition

Leaf discs of 6 millimeter (mm) diameter were cut from each leaf samples using a sharp device (Single Hole Punch). Leaf discs was then transferred onto a microscope glass slide and carefully placed into the base of acetone vaporizer (Perm-O-Fix, Atlanta Lab Systems, LLC) and subjected to discharge of 1 drop of acetone. The glass slide was warmed up for the acetone to dry up before Triacetin (EMSL Analytical Inc.,) was added. A drop of Triacetin was added to discs instead of performing leaf staining to avoid confusion on detection of particles deposition on the leaf surface. The filter clearing method was used instead to detect deposition of particles on internal parts of the leaf surface. Leaf particles deposition on the outer leaf surface was of secondary importance on this part of the analysis. Leaf discs were then set for 24 hours to facilitate leaf clearing prior to particle counting. A camera (MA 1000, Amscope, Irvine, CA) mounted to a compound microscope (T690-C, Amscope, Irvine, CA) was used to investigate number of particles deposited in a given leaf surface area at 10 x 10 magnification.

Stomatal Imprints

Five leaf discs were cut and prepared from each plant species except for conifers where leaf fascicle was obtained from each needles for stomatal imprints. A base or top coat polish (Nail Treatment, Beauty 21 Cosmetics, Ontario, CA) was used as adhesive and solution to

adhere to leaf surface for a maximum of 30 minutes to completely cover the entire area. After thorough drying, the hardened solution in a form of a film was carefully peeled and transferred to an adhesive tape with the abaxial surface facing the sticky side of the tape prior to mounting in a microscope glass slide cover. The imprints were subjected to observations using the compound microscope at 40 x 10 for SD and SP. The SPS (um^2) was measured through length (L) and width (W) values, assuming an elliptical shape of the pore (Adriaenssens, et. al., 2012). The number of stomata was counted in a given microscopic field area of the leaf to calculate stomatal density.

Statistical Analysis

All data were analyzed using the SAS statistical software (Version 9.2 (32) Cary, NC). Significant difference on particulate matter loading (g/cm^2), dust deposition on leaf cellular surfaces and stomatal characteristics were determined through one-way analysis of variance. Test of least significant difference was performed when the F-test was significant ($P < 0.05$). Data was pooled after a significant ANOVA with Tukey HSD procedure to compare difference on means.

Results and Discussion

Dust loading varied significantly among each species (Table 1). At PM_{20} sizes, a significant statistical difference ($p<0.0001$) exists. Highest particulate matter loading was observed in Yaupon and Arborvitae plants (0.00025 g/cm^2) followed by Eastern red cedar (0.00012 g/cm^2), Arizona cypress (0.00002 g/cm^2), and American holly (0.00001 g/cm^2). At PM_8 sizes, no significant difference on particulate matter was observed ($p=0.0516$). Yet on $PM_{2.5}$ sizes, highest dust loading (0.00036 g/cm^2) was also observed in Arborvitae plants but fewer particles retained inside the leaf cuticle (Figure 2.) and was significantly different ($p=0.0002$) from other four plant species. Total surface area of conifer foliage was higher than deciduous plants (Matyssek et al., 1995), therefore has more potential to adsorb dust on leaf surface which was consistently observed in Arborvitae from $PM_{2.5}$ to PM_{20} than other conifers used in the study. Adrizal et al., (2008) identified conifers (e.g. Norway spruce) to significantly hold more mass of PM_{10} due to needle arrangement. Khan & Abbasi (1999) point out that smaller leaves are efficient particle collectors than larger leaves supporting higher dust (e.g. PM_8, PM_{20}) accumulated in Yaupon plants.

Table 1. Mean values of particulate matter loading in three particles sizes of five plant species quantified through gravimetric analysis. Different letters indicate significant differences.

Plant species	Particulate Matter Size (g/cm^2)		
	$PM_{2.5}$	PM_8	PM_{20}
American holly	0.00009 [b]	0.00005 [b]	0.00001[b]
Arborvitae	0.00036 [a]	0.00026 [a]	0.00025 [a]
Arizona cypress	0.00017[b]	0.00012 [ab]	0.00002[b]
Eastern red cedar	0.00007[b]	0.00007 [ab]	0.00012 [ab]
Yaupon	0.00006[b]	0.00014 [ab]	0.00025 [a]

Table 2 shows particulate matter deposition through particle counting using a microscope at 1000x magnification which yield a significant statistical results among all five species at particulate sizes of $PM_{<2.5}$ ($p=0.0211$) and $PM_{>5,<10}$ ($p=0.0173$). No significant statistical difference was observed in $PM_{>2.5, <5}$ ($p=0.4710$) and $PM_{>10}$ ($p=0.1307$) sizes. At

$<PM_{10}$ size division, the number of particles counted in Arizona cypress (Figure 3.a.) (160.0 $PM_{<10}/mm^2$) was significantly high compared to Eastern red cedar (95.3 $PM_{<10}/mm^2$) and American holly plants (64.3 $PM_{<10}/mm^2$), Arborvitae (34.7 $PM_{<10}/mm^2$) and Yaupon (23.1 $PM_{<10}/mm^2$) plants. Arizona cypress, American holly and Eastern red cedar trapped more particles from $PM_{<5}$ to $PM_{<10}$ than other species. This was supported by a study of Joshi & Bora (2011) identifying leaf characteristics with waxy coating and rough surface to accumulate more dust on its leaf surface. At $PM_{<2.5}$ size division, Yaupon plants (176.0 $PM_{<2.5}/mm^2$) were detected to accumulate more particulate matter (Figure 4.a.) followed by American holly (51.8 $PM_{<2.5}/mm^2$), Eastern red cedar (45.4 $PM_{<2.5}/mm^2$), Arizona cypress (32.5 $PM_{<2.5}/mm^2$) and Arborvitae (14.5 $PM_{<2.5}/mm^2$), respectively. Large-leaved species are less effective barriers against finer dust which can travel greater distances (Prajapati, 2012). As opposed to prior findings, this can explain Yaupon plants quantified with most number of particles of sizes greater than $PM_{2.5}$ but less than PM_5.

Table 2. Mean values of particulate matter in four particles sizes (+ s.d.) quantified through microscope optical counting. Different letters indicate significant differences.

Plant species	Number of Particles in a leaf area (particles/mm^2)			
	$PM_{<2.5}$	$PM_{<5}$	$PM_{<10}$	$PM_{>10}$
American holly	51.8 ± 54.5 [ab]	127.0 ± 118.0 [a]	64.3 ± 78.8 [ab]	7.7 ± 8.7[a]
Arborvitae	14.5 ± 18.9 [b]	29.9 ± 7.2 [a]	34.7 ± 29.3 [b]	1.9 ± 2.6 [a]
Arizona cypress	32.5 ± 36.8 [b]	156 ± 129.0 [a]	160.0 ± 99.8 [a]	19.2 ± 32.1 [a]
Eastern red cedar	45.4 ± 98.8 [ab]	41.6 ± 42.4 [a]	95.3 ± 53.0 [ab]	18.5 ± 25.3 [a]
Yaupon	176.0 ± 110.3 [a]	149.0 ± 114.0[a]	23.1 ± 23.8 [b]	3.8 ± 6.3 [a]

There was a significant statistical difference in stomatal density among five plants (Table 3). Yaupon's frequency of stomata (Figure 4.b.) was significantly high compared to four other plants (p=0.0001). The number of closed and open stomata in Arizona cypress plants (Figure 3.b.) was also statistically different among species (p< 0.0001). All stomata were observed closed upon inspection in the microscope. Stomata that were obstructed and covered with particulate matter were also identified as closed stomata. In Singh & Sinha (2010) study, dust deposited on the leaf surface clogged stomata which risks to inhibit physiological function of plants. Stomatal length (p=0.3969) and stomatal pore size (p=0.1187) showed no significant statistical difference (Table 4).

Table 3. Stomatal characteristics (stomatal density (SD), closed stomata, open stomata) of five plant species as inspected through microscope optical counting. Different letters indicate significant differences.

Plant species	Particulate Matter Size				
	Closed stomata	%	Open stomata	%	Total stomata
American holly	0.80 [b]	3.98	16.6 [b]	96.02	17.40 [b]
Arborvitae	2.60 [b]	18.64	10.4 [b]	81.36	15.00 [b]
Arizona cypress	12.20 [a]	100	0 [a]	100	12.20 [b]
Eastern red cedar	0.80 [b]	6.88	10.40 [b]	93.12	11.20 [b]
Yaupon	5.80 [b]	18.71	22.60 [b]	81.29	28.40 [a]

Table 4. Stomatal features [stomatal length (SL), stomatal pore surface (SPS)] of five plant species as inspected through microscope optical counting. Different letters indicate significant differences.

Plant species	Stomatal features	
	Stomatal length	Stomatal Pore surface
American holly	0.005 [a]	0.000016 [a]
Arborvitae	0.019 [a]	0.000007 [a]
Arizona cypress	0.009 [a]	0.000000 [a]
Eastern red cedar	0.006 [a]	0.000017 [a]
Yaupon	0.003 [a]	0.000003 [a]
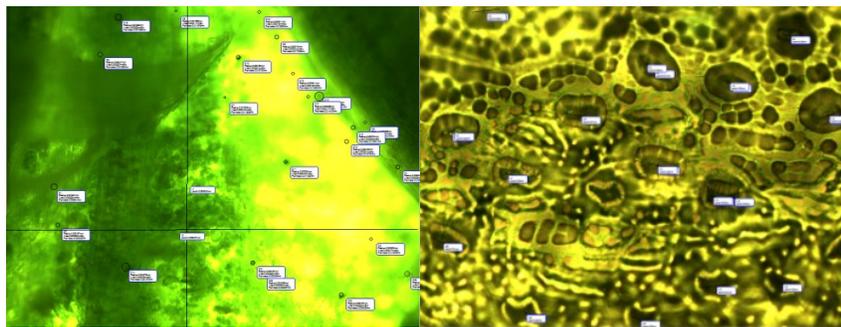
Figure 2. Dust deposited on intercellular surface of Arborviate upon microscope inspection at 10x magnification and stomatal characteristics at 40x magnification.
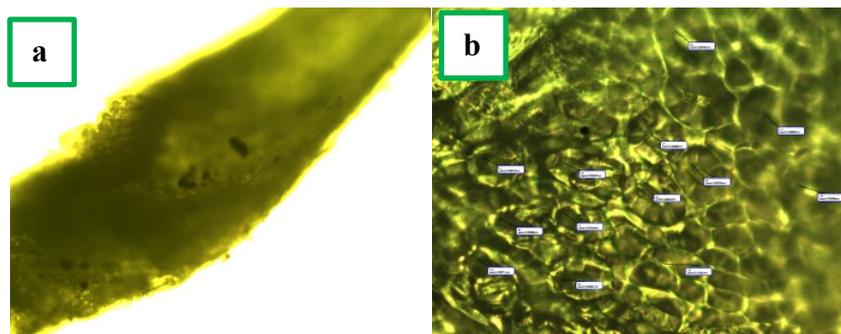
Figure 3. a.) Dust deposited on Arizona cypress plants upon microscope inspection at 10x magnification and b.) stomatal characteristics at 40x magnification.
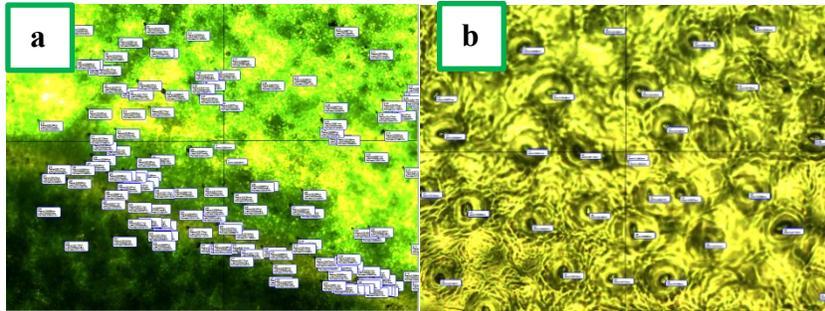
Figure 4. a.) Dust deposited on Yaupon plants upon microscope inspection at 10x magnification and b.) stomatal characteristics at 40x magnification.

Conclusion

Based on the results of the study, the following can be concluded:

1. The gravimetric analysis used to determine particulate matter loading can be used in future study in determination of an effective plant to be used as shelterbelts in poultry farms to trap and control dust dispersion.
2. Yaupon plants effectively trapped larger size of particulates of PM_{20}. Arborvitae significantly collected both small and large sizes of particulates of $PM_{2.5}$ and PM_{20}.
3. Inspection through optical counting showed highest stomatal density of $PM_{2.5}$ in Yaupon plants which can be either be associated to its specific physiological trait or its adaptive response to particulate exposure (Ogunkunle et al., 2013).
4. Arizona cypress deposited particulates of sizes $PM_{>5,<10}$, significantly resulting to blocked stomata which can further inhibit gas exchange essential physiological function of plants.
5. Stomatal length and pore space do not significantly differ among each plant; therefore adverse effects from direct exposure to dust from poultry emission can't be assessed.

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Appendix C – Draft of Paper: Atmospheric Ammonia Effects on Foliar Ammonia Content and Physiological Response of Plants

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Abstract

Six plant species comprised of Yaupon, Eastern red cedar, American holly, Arizona cypress, Arborvitae and Roughleaf dogwood were utilized to determine plant species' effectiveness in removal of atmospheric ammonia. Plant species were exposed to various levels of ammonia (e.g. 1, 5 & 10 ppm) in a laboratory-controlled setup and foliar ammonia content was analyzed and quantified using an enzymatic technique. Statistical difference showed significant difference on foliar ammonia content ($p<0.0001$) with Eastern red cedar measured with the highest foliar ammonia content. The effect of NH_3 exposure on leaf gas exchange was evaluated through physiological response of plants. All three factors of species, treatment and exposure (before and after) significantly impacted net photosynthetic rate (A_n) ($p=0.0057$) and stomatal conductance (g_s) ($p=0.03$) of plants. Interactions of *species x treatment and species x exposure* ($p<0.0001$) on transpiration rate were significant, but not on the three factor interactions ($p=0.07$).

Keywords: enzymatic technique, foliar ammonia, physiological response, plants

Introduction

Highly variable combinations of multiple pollutants in ambient atmosphere can pose negative effects on vegetation (Krupa & Legge, 2009) through transfer by the combined forces of diffusion and flowing air movement (Khan & Abbasi, 1999). Vegetation constantly exposed to the atmospheric pollutants may absorb, accumulate and integrate pollutants impinging on the foliar surfaces. Effects are primarily reflected in the plant physiology on major system and organs of plants constantly exposed to the atmosphere where continuous exchange of gases in and out of the environment occurs (Radhapriya et al., 2012). A number of agricultural chemicals adversely affect growth and development of plants (Furlan et al, 1999). Among several gases measured in confinement poultry houses, ammonia (NH_3) and dust were primary contaminant of concerns at present average airborne concentrations (Adrizal et al, 2008).

Ammonia (NH_3) is a colorless gas at room temperature but becomes a liquid when compressed. It easily dissolves in water and has a pungent and suffocating odor with odor threshold at 5 ppm. It reacts with strong oxidizers, acids, halogens, bleach, salts of silver, zinc, copper, and other heavy metals which makes it corrosive to copper and galvanized surfaces. A major use of it includes refrigeration, plastic and textiles processing but aqueous solution are commonly used as household cleaning agents (Salocks & Kaley, 2003). In the atmosphere, it is the third most abundant nitrogen form which can increase more rapidly due to various natural and anthropogenic sources (Wollenweber & Raven, 1993). Agriculture is the most important source for NH_3 emissions mainly resulting from livestock management and to a minor part from fertilizer application while industry, power plants, traffic, human excreta and other sources only a minor role (Fangmeier et al., 1994).

Serious dieback of forests and decline in number of plant species are observed in the Netherlands where intensive animal breeding is concentrated (Roelofs, 1986). Van Hove et. al., (1990) mentioned that NH_3 is toxic in plants as it also functions as an electron acceptor causing uncoupling of electron transport in the membrane and by saturating membrane lipids causing membrane dysfunction. Though generally, it is the assimilation capacity of the plant species that determines the degree of injury. If the assimilation capacity is not sufficient to detoxify NH_y, acute (visible) injuries may occur (Fangmeier et. al., 1994). However, sensitivity of terrestrial plants to NH_3 exposure varies; the capacity of some plants to detoxify NH_3 upon absorption is entirely dependent on the availability of carbohydrate (Dueck et al., 1998).

Scots pine enclosed in a chamber with NH_3 has lower needle water potential, increasing drought sensitivity of the plant (Dueck et al., 1998). Honey locust's color was enhanced and

lower damage values were recorded and significant increase of nitrogen in the leaves indicates greater metabolism or detoxification of the absorbed NH_3 compared to red cedar, hybrid poplar and reed canary grass (Adrizal et al, 2008). Wollenweber & Raven (1993) reported that atmospheric NH_3 increased the growth rate of perennial ryegrass (*Lolium perenne)* but not on its nitrogen acquisition per unit dry matter with increasing gaseous NH_3 concentration.

Though there have been several studies on deposition of air pollutants to vegetation, few has been known to determine the effectiveness of trees on mitigating air emissions from poultry buildings. This study focused on the effectiveness of several species of trees on removing NH_3, one of the major pollutants in poultry buildings. Plant species that have higher foliar ammonia content might be effective in withstanding poultry farms' environmental conditions.

Materials and Methods

Exposure to Ammonia. Seedlings of six different plant species comprised of 12-months-old *Ilex vomitoria (evergreen shrub)*, 14-months-old *Juniperus virginiana (evergreen conifer)*, 10-months-old *Ilex opaca (broad-leaved evergreen)*, 12-months-old *Cupressus arizonica (evergreen conifer)*, 12-months-old *Thija plicata x standishii* (evergreen conifer) and 15-months-old *Cornus drumondii (deciduous shrub),* moderately to fast growing trees were selected as test species for the experiment. The seedlings (20-50 cm; 6-14 months old) were obtained from commercial sources; selected for uniform size, and individually planted into one-gallon polypropylene pots. Seedlings were housed and allowed to adjust to greenhouse conditions for four months prior to start of ammonia exposure and measurements. The planting medium consists of a potting soil mixture with slow release of macro and micro mineral nutrients (Hyponex by Scotts ©Potting Soil). All plants were watered to field capacity as needed before exposure to different ammonia levels to minimize stress due to greenhouse condition. Throughout the experiment, each pot was watered twice per week or whenever the soil volumetric water content becomes less than 10% using a soil moisture meter (Field Scout TDR 100/200, Spectrum Technologies, Plainfield, IL). During the exposure, plant pots were sealed with Parafilm sheets to avoid NH_3 deposition on the soil which might affect apoplastic NH_4^+ assimilation through the roots.

A controlled-chamber (28-Liter capacity) was made up of Plexiglas material (1.333ft x 1ft x 2.1667 ft.) and constructed and designed systematically to ensure containment of NH_3 and prevent escape of the gas. The chamber was housed inside the greenhouse to reflect ideal ambient conditions and primarily to ensure stomatal opening during the experiment. Several inlets were drilled that served as inlet and pathway for NH_3 injection point and an outlet for an NH_3 detector tube to determine concentration inside. Inlets were covered with rubber stopper and supplemented with Parafilm tape to prevent wall losses. Inner walls and other supplementary instruments were coated with Teflon material to avoid ammonia reaction with the surface. Air circulation in the chamber was facilitated by an axial fan mounted through a 1" stainless steel rod attached inside the chamber. The fan was kept in continuous operation to reduce space variability of gas concentration and reduce the boundary resistance layer of the leaf and to avoid a critical wind velocity (0.3 – 0.4 m s[1]) (Van Hove et.al., 1989).

Preliminary experiment was performed to ensure leaf damage will be prevented due to air velocity and was monitored using an air velocity meter (Model 9545-A, TSI Inc., Shoreview, MN). Measurement were done 10 and 15 inches above surface floor of the controlled-chamber which covers the range of seedlings height, measured from the pots going upwards to leaf foliage surface. Temperature, relative humidity and light intensity were also monitored throughout the experiment using the Hobo Data logger sensors. The chamber was tested for leak test using the Nextteq Irritant Smoke Tube Kit (P/N 9501, Nextteq LLC, Tampa, FL) to check if gas escaping the chamber was present. The test was performed and a considerable waiting period (e.g. 15 minutes) was observed for the smoke to cover the entire chamber before

the axial fan was operated to run and was opened after observation that no smoke was coming out. A one-hour exposure for sampling duration was also determined after the preliminary experiment after closer values between 30 minutes and 60 minutes of exposure was achieved in the chamber. Plants were exposed to different levels of ammonia to increasing treatment levels from low (1ppm/hour), medium (5 ppm/hour) to high (10 ppm) treatment level. After exposure duration, fresh plant foliage were randomly collected from each replicate species and subjected to laboratory analysis for foliar ammonia content at the Environmental Assessment Laboratory. Foliar ammonia was determined through a modified enzymatic technique (Kun & Kearney, 1974).

Ammonia Quantification. Leaf samples were thoroughly washed with water, 0.1 M HCl and 0.2% detergent solution to remove waxy/greasy coating on the leaf surfaces. Samples were then dried with tissue paper before drying in the oven at $70^{\circ}C$ for 48 hours to remove moisture, render plant tissues inactive and stop enzymatic reactions. Drying plant material was necessary to reduce risk of degradation and other chemical changes during storage (Marur & Sudek, 1995). After drying, leaf samples were brittle enough to be grounded with cooled mortar and pestle (Mattson et. al., 2009) to a powdery consistency and stored until ready for extraction process. Oven-dried leaf samples (500 mg) were suspended in 10% Trichloroacetic acid solution to deproteinize (Ali & Lovatt, 1995) and stored at -20°C to prevent degradation of leaf samples especially when analysis wasn't performed immediately. Leaf samples undergo centrifuge processing at 4100 revolutions per minute (rpm) at 4°C for 10 minutes to liberate NH_3 from the extract. The homogenate (extracts separated from the solid particles) were neutralized using 2 M of $KHCO_3$ to attain dynamic equilibrium between NH_3 and NH_4. Neutralized extract sample was then prepared for enzymatic analysis. The following reagents were pipetted into the sample cell (1 cm diameter or sample cell light path) a.) 200uL of 0.5M tris-HCl buffer (pH 8); b.) 100uL of 0.1M 2-oxoglutarate solution (pH 7.4); c.) 200uL of 8mM β-NADH solution; d.) 2000uL of distilled water; and e.) 100uL of neutral extract sample. The absorbance (A1) of the solution was recorded after two minutes in the spectrophotometer (DR 3900 Benchtop Spectrophotometer, Hach Company, Loveland, CO). The 200µL of glutamate dehydrogenase (GLDH) enzyme which was commercially purchased was added to the solution to start the reaction, and the absorbance (A2) was recorded immediately. Calibration curve was generated using NH_3 standard dilution to have six ammonia concentrations. Absorbance was measured using the same enzymatic procedures. Foliar ammonia content was then calculated using the resulting calibration curve.

Statistical Analysis

The effectiveness of various tree species on foliar NH_3 uptake was determined with two-way analysis of variance procedure using SAS statistical software (Version 9.2, SAS, Cary, NC). Tukey's test was performed after significant ANOVA to see differences on the means.

A repeated measure design with one fixed among subjects (species) and two fixed factors within subject (treatment levels, exposure) was used to determine significant difference on foliar NH_3 content of plants. Repeated measure analysis of variance (ANOVA) was performed on the means to identify treatment and exposure effects on the physiological responses of six species.

Results and Discussion

Foliar NH₃ content

The pooled mean of the foliar NH_3 concentrations (µmol NH_3/ml of leaf extracts from dry weight leaf samples) of six plant species are shown in Figure 1. Results showed a significant statistical difference (p<0.0001) on foliar NH_3 content of all six species of plants. Eastern red

cedar had the highest foliar NH_3 content (0.26 µmol/mL) followed by American holly (0.24 µmol/mL), Arizona cypress plants (0.23 µmol/mL), Roughleaf dogwoods (0.22 µmol/mL), Arborvitae plants (0.19 µmol/mL) and Yaupon (0. 14 µmol/mL), respectively.

Adrizal et al. (2008) found higher capacity of deciduous trees over evergreens (e.g. hybrid poplar, honey locust, and reed canary grass) to incorporate NH_3 into their tissue and plant foliage to trap approximately 30% of NH_3 discharged from the exhaust fans of poultry and livestock barns. Adriaenssens et al. (2010) found that NH_3 uptake was always higher for deciduous species than for pine species (e.g. potted silver birch, European beech, pedunculated oak and Scots pine saplings). Results of this study however did not follow the same conclusions reached by Adrizal et al. (2008) and Adriaenssens et al. (2010). Eastern red cedar which is a conifer yields the highest NH_3 content compared to Roughleaf dogwood plants. Adrizal et al. (2008) mentioned that leaf surface area between broad-leaf and needle leaf can be a factor for this higher efficiency of conifers to absorb NH_3-N. Adriaenssens et al. (2010) related this to lower N demand of conifers because of lengthy needle retention and efficient internal N recycling as an attributing factor. For plants measured with lower foliar NH_3 content (e.g. Yaupon, Arborvitae), assimilation rate might have been limited due to compensation capacity of plants. Langford & Fehsenfeld (1992) found out in wider perspective when gaseous NH_3 above a montane-subalpine forest in Colorado becomes either a source or sink depending on atmospheric conditions. In a laboratory experiment, increased temperature caused the plant to switch from being a strong sink for atmospheric NH_3 to being a significant source (Husted & Schjoerring, 1996).
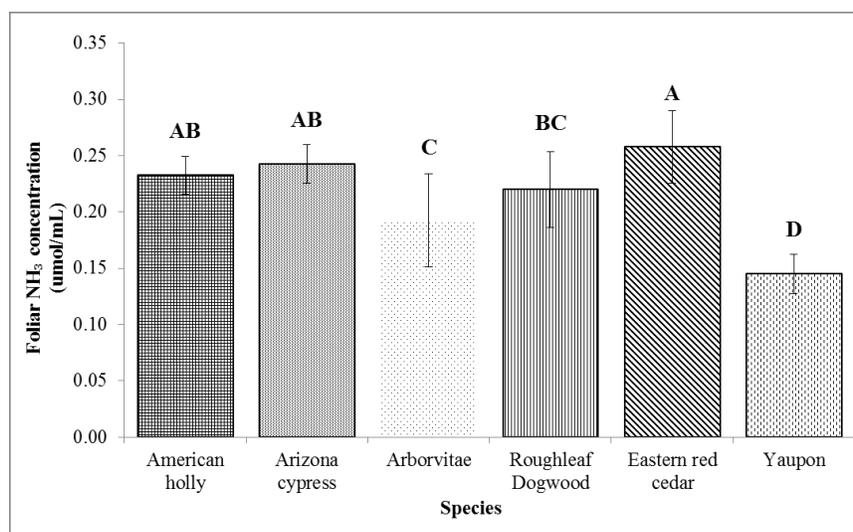
Figure 1. Comparison of means of foliar NH_3 content of the six plant species among all treatment levels. Error bars represent standard deviation. Groups with the same letter are not statistically different.

Figure 2 shows significant statistical difference (p=0.0006) of treatment levels on the foliar NH_3 content of the plants as assimilated by the leaves. Ammonia concentration of plants species not exposed to NH_3 (0.19 umol/mL) were significantly lower when compared to plants exposed at three treatment levels (e.g. 0.221 umol/mL, 0.224 umol/mL, 0.225 umol/mL). Both species x treatment interaction significantly influenced foliar NH_3 content (p=0.0137).

Regardless of treatment levels, exposure of NH_3 resulted to initiate the same effect on increased foliar NH_3 content of plants. Obvious increased on foliar NH_3 content on control and plants subjected to treatment was observed on Yaupon, Arborvitae and Roughleaf dogwood plants (Figure 3).
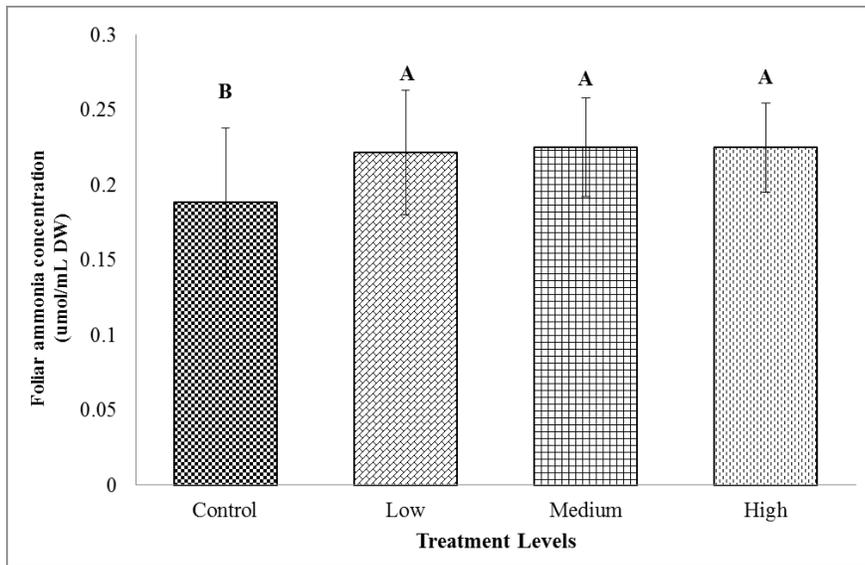
Figure 2. Tukey's test in the determination of differences on foliar NH$_3$ content among treatment levels. Groups with the same letter are not statistically different at the 5% level.
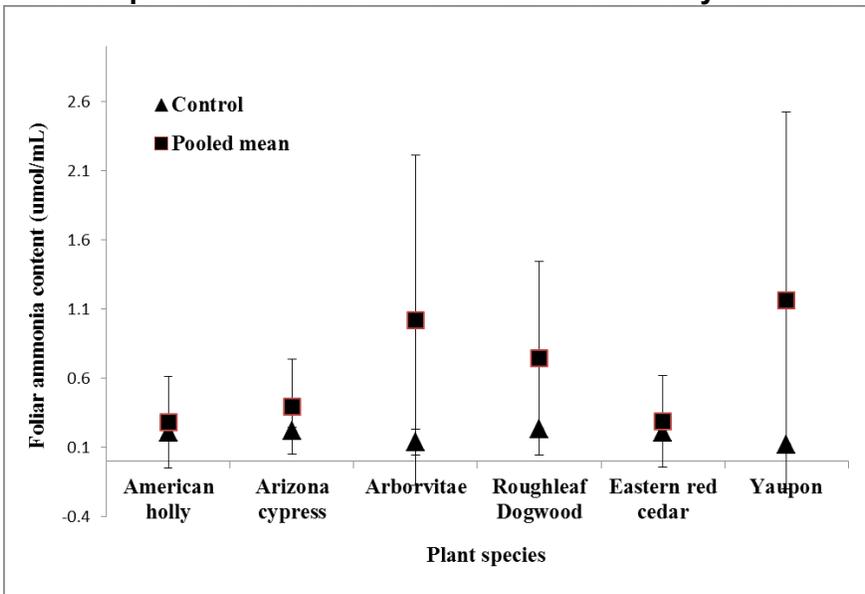
Figure 3. Pooled foliar ammonia content at three treatment levels in comparison to control plants. Error bars represent standard deviation.

Treatment levels also affected the foliar ammonia concentration as plants not exposed to ammonia were analyzed with lower ammonia content ($p<0.0001$) than those exposed at various concentration.

Physiological response: Net photosynthetic rate, stomatal conductance, transpiration rate

Leaf photosynthesis (A_n) varies with species type ranging from 2 to 17 µmol CO_2 m^{-2}s^{-1} for conifers, 3-22 µmol CO_2 m^{-2}s^{-1} for evergreen broad-leaved plants and highest for deciduous broad-leaved plants at 3-27 µmol CO_2 m^{-2}s^{-1} (Raghavendra, 1991). Unlike Adriaenssens et al.

(2010) study, the results showed high A_n on most conifer and evergreen plants (e.g. Arizona cypress, American holly) than deciduous plant species (e.g. Roughleaf dogwood) used for the study. This might indicate, according to Raghavendra (1991), that evergreen needle-leaf trees are efficient in light capture, thus reducing energy waste especially during light saturation.

Change on the net photosynthetic rate of plant species after exposure to NH_3 increase and decrease response but no distinct response from low, medium to high treatment level (Figure 4a, b, c). Interactions of species vs. treatment levels ($p<0.0001$) and species vs. exposure ($p=0.0056$) have a significant difference on plants response (A_n) at three treatment levels.

American holly plants' A_n decreased after exposure to NH_3 at all treatment levels while five other plants have inconsistent response at different treatment levels. Eastern red cedar which is a conifer and deciduous broad-leaf Roughleaf dogwood plant both responded with decreased A_n at low and high treatment level. Regardless of the response, Eastern red cedar's A_n values was still in the expected range of 2-17 μmol CO_2 $m^{-2}s^{-1}$ but an alarming indicator for Roughleaf dogwood with all values at three treatment levels falling below its A_n. Pearson & Stewart (1993) mentioned that NH_3 does not adversely affect photosynthesis, in fact increases CO_2 uptake. A study supporting the latter findings (Van Hove et al., 1990) indicating an increased CO_2 assimilation in poplar leaves after exposure at 144 ppb for 6 to 8 weeks which can be associated to the required carbon skeletons from carbohydrates synthesized during photosynthesis which is an essential component for amino acids production (Massad et al., 2008). Increased values of A_n of some plants have suggests a good response as photosynthesis is the only requirement for growth of plants, particularly on productivity. However, as stomata constantly take in more CO_2, it also lets in more NH_3 inside making it vulnerable to other pollutants in real field conditions (Raghavendra, 1991).

Stomatal conductance of the lower surface of the leaf has a representative conductance value of 0.02-0.12 mol H_2O $m^{-2}s^{-1}$ for trees and 0.08-0.40 mol H_2O $m^{-2}s^{-1}$ for crops. For open and large area stomata, representative conductance value can be as high as 0.76 mol H_2O $m^{-2}s^{-1}$ but only 0.07 mol H_2O m^2s^1 for small open stomata. All throughout the leaf parts, open stomata of trees and xerophytes can be in the range of 0.04 to 0.16 mol H_2O $m^{-2}s^{-1}$ (Raghavendra, 1991). Figure 5(a, b, c) shows the stomatal conductance before and after exposure to NH_3 at all treatment levels. As observed in net photosynthetic response of plants, there's no consistent pattern or trend of increasing or decreasing response on stomatal conductance of plants at increasing treatment levels. Yet, species vs. treatment levels and species vs. exposure interactions both have a significant statistical difference ($p<0.0001$) on plants response. As a result, all three factors significantly ($p=0.03$) affected stomatal response of plants.

Stomatal conductance (g_s) of Arizona cypress and American holly plants were unaffected by the concentration level, i.e. no matter what the treatment level was, the response was constant. Both plants responded and exhibited increased stomatal conductance at all treatment levels which could reflect higher intake of NH_3 into the leaf. Van Hove & Bossen (1994) had the same observation on Douglas fir plants when exposed to low concentrations of NH_3 but with control on light intensity. However, the interference and effect of light on plants on this study has been considered and amount of light were kept constant (1200 umol CO_2 $m^{-2}s^{-1}$) in the leaf-gas exchange system. Hanstein et al. (1999) also observed g_s to increase with increasing NH_3 concentration (0 - 30 nmol/ mol of air) on three native meadow grasses species and poplar leaves after exposure at 144 ppb for 6 to 8 weeks (Van Hove et al., 1990).
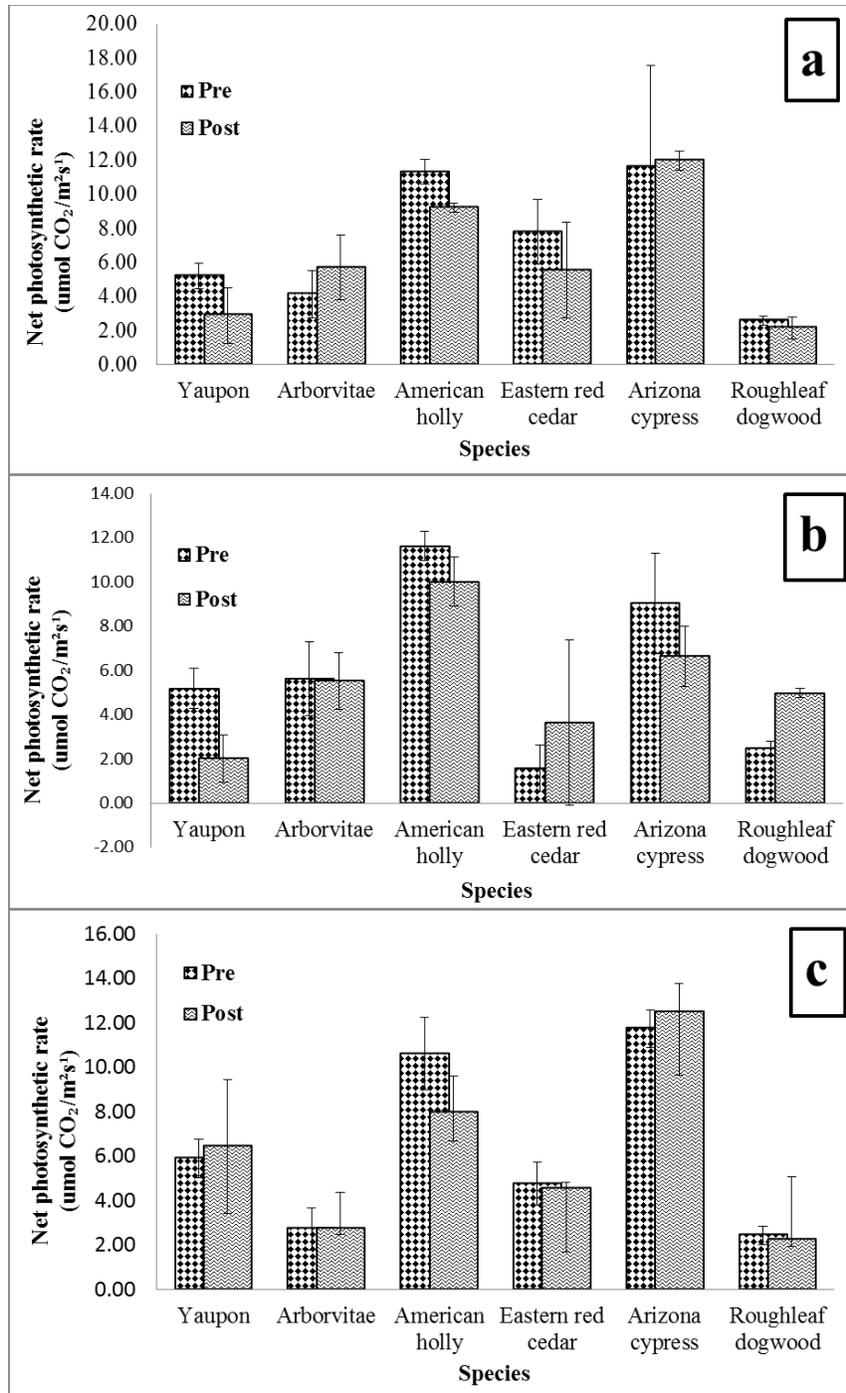
Figure 4. Comparison of net photosynthetic rate of six plant species measured using LiCOR 6400 prior to and after exposure to NH$_3$ at a.) low, b.) medium, and 3.) high treatment levels. Error bars represent standard errors.

Evergreen conifer needles have generally lower g$_s$ than do broadleaf plants (Matyssek et al., 1995), therefore explaining the lower stomatal conductance of Yaupon and Arborvitae which were evergreen and conifer. Despite decreased response of plants at some treatment levels (e.g. Arbovitae, Yaupon), g$_s$ values were in representative values for both large and small open stomata. Even If g$_s$ on the lower leaf surface were to be reflected, regulated g$_s$ values still lies

within the expected range (e.g. 0.02-0.12 mol H_2O $m^{-2}s^{-1}$ for trees) but not for Roughleaf dogwood after exposure at high treatment level (0.01 mol H_2O $m^{-2}s^{-1}$).

Transpiration rate (E) after exposure to NH_3 treatment levels showed either an increased or decreased response of plants (Figure 6a, b, c). Transpiration rate (E) values is low at 0.8 mmol $m^{-2}s^{-1}$ if photosynthetic photon flux density (PPFD) or light energy for plants is low though it can gradually increase if PPFD becomes medium (2.5 mmol $m^{-2}s^{-1}$) and high (5 mmol $m^{-2}s^{-1}$). Interactions of species vs. treatment levels and species vs. exposure yielded significant statistical difference ($p<0.0001$) on transpiration rate of plants but not on all three factors ($p=0.07$).

Usually increased E values are dictated by absence of water stress in the plants and decreased E when water supplies in intercellular surfaces are limited (Raghavendra, 1991). In this study, amount of water vapor delivered into the leaf's intercellular surface was controlled. Despite lower E values per unit of leaf surface for loblolly pine than broad-leaf species, E values per seedling of similar plant size was greater for pine because of its greater total leaf surface (Kramer, 2012) but this wasn't observed in one out of three conifers used in the experiment. Eastern red cedar plants constantly resulted in decreased (E) values at all treatment levels of NH_3 (9.50 to 2.97 mmol $m^{-2}s^{-1}$). In general, the control on ΔE rests with stomata (Nobel, 1999) and the function of stomatal closure highly influenced E status especially by mid-day when stomata closed as a response to increasing temperature. In a study on two species of olive trees, Lo Bianco & Avellone (2015) observed (E) from 0.3 to 1.6 mmol $m^{-2}s^{-1}$ throughout the sampling duration (08:00-19:00) with peak measurement at noon in *Biancolilla* species. *Cerasuola* species was observed with 0.3 to 1.2 mmol $m^{-2}s^{-1}$ (E) but had a different peak time (13:30). However, since light intensity were taken into account to play a major role in plants E, an established PAR of 1200 umol m^2s^1 was set constant and applied for all the gas-exchange measurements on all plants. Even if the response was inconsistent for Arizona cypress and Arborvitae, results showed higher E values than high PPFD at 5 mmol $m^{-2}s^{-1,}$ specifically for Arizona cypress as reflected at all treatment levels.
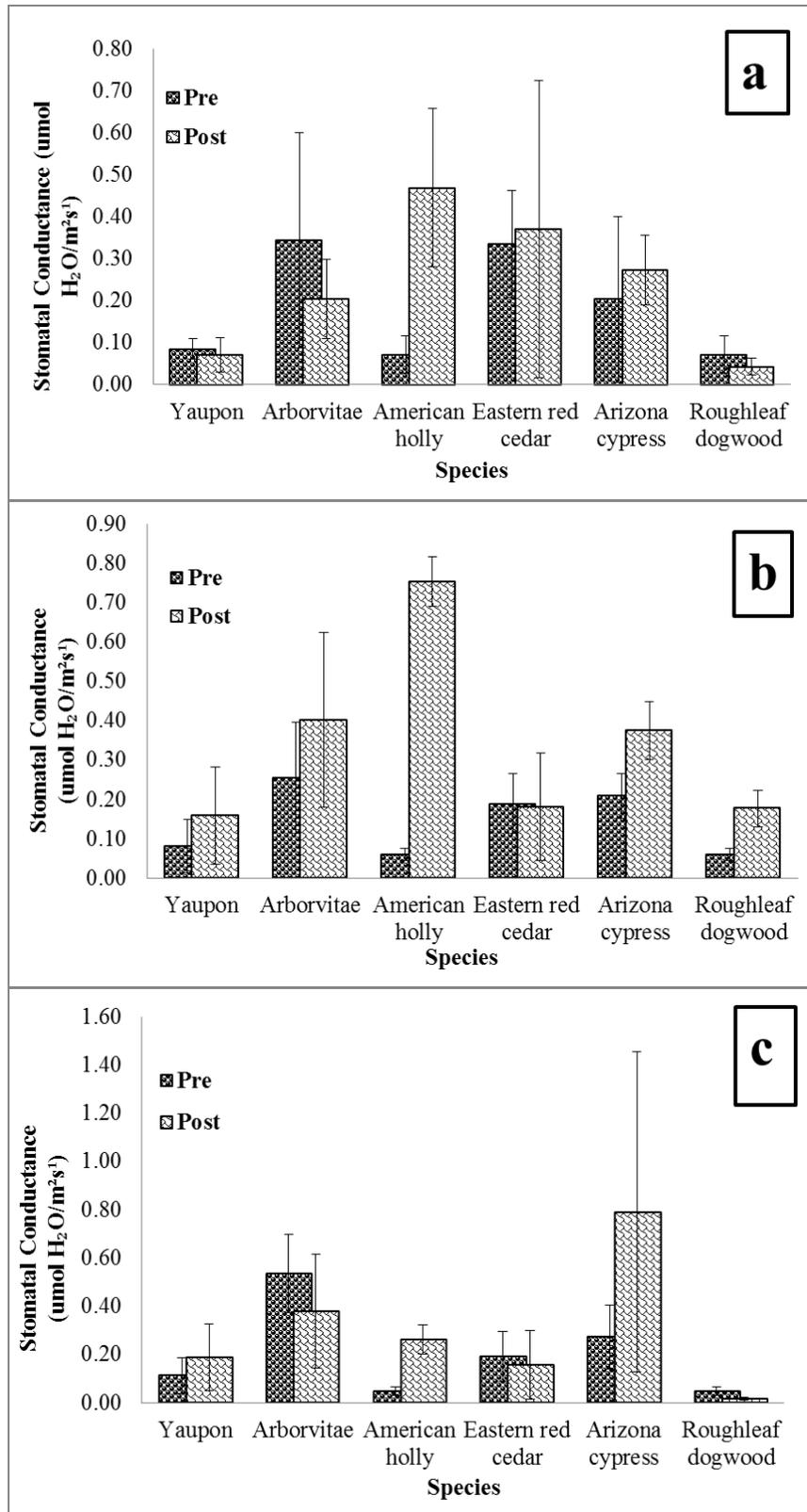
Figure 5. Comparison of stomatal conductance of six plant species measured using LiCOR 6400 prior to and after exposure to NH$_3$ at a.) low, b.) medium, and 3.) high treatment levels. Error bars represent standard errors.
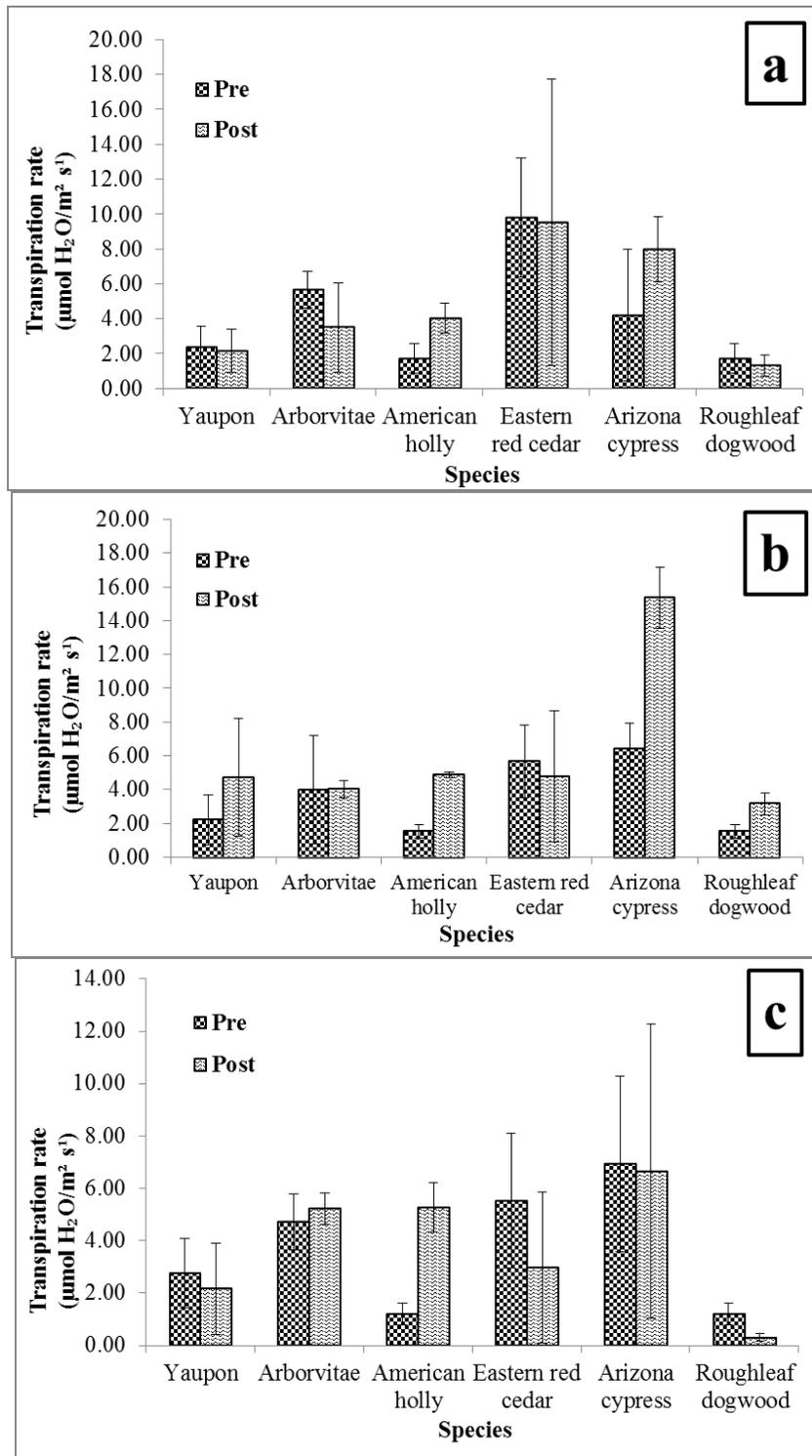
Figure 6. Comparison of transpiration rate of six plant species measured using LiCOR 6400 prior to and after exposure to NH$_3$ at a.) low, b.) medium, and 3.) high treatment levels. Error bars represent standard errors.

Conclusion

Based on NH_3 assimilation, Eastern red cedar will be the best option for shelterbelt design followed by Arizona cypress, American holly, Roughleaf dogwood, Arborvitae and Yaupon.

Exposure to NH_3 significantly lowered the net photosynthetic rates of the three plants analyzed with higher NH_3 content (Eastern red cedar, Arizona cypress and American holly). Yet, all values were still within the range expected for conifers (2 - 17 µmol CO_2 $m^{-2}s^{-1}$) and evergreen plants (3 - 22 µmol CO_2 $m^{-2}s^{-1}$). Therefore, A_n, which is essential to plant metabolism (Fitter & Hay, 2002), will not be limited. Exposure to NH_3 caused increased stomatal conductance (g_s) and transpiration rate (E) on American holly and Arizona cypress. In dry and drought conditions and when NH_3 concentration is elevated, both plants will be at risk for higher potential for water loss as water relations will be altered and recovery from water loss will be difficult. Therefore, under these conditions, American holly and Arizona cypress will not be good choices for a shelterbelt to control NH_3.

Acknowledgement

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Figure 2. Measurement of physiological parameters using LiCOR 6400 instrument.

Figure 3. (a) Homogenized leaf samples, (b) Neutralized leaf extracts, (c) Tissue extracts for spectrophotometric measurement, (d) UV-VIS DR 3900 spectrophotometer.

Figure 1. American holly and Roughleaf dogwood plants exposed to ammonia in a controlled-chamber.

Figure 4. Comparison of means of foliar NH_3 content of six plant species among all treatment levels. Error bars represent standard errors.

Figure 5. Comparison of net photosynthetic rates of six plant species measured using LiCOR 6400 prior to and after exposure to NH_3. Error bars represent standard errors.

Figure 6. Comparison of stomatal conductance of six plant species measured using LiCOR 6400 prior to and after exposure to NH_3. Error bars represent standard errors.

Figure 7. Comparison of transpiration rates of six plant species measured using LiCOR 6400 prior to and after exposure to NH_3. Error bars represent standard errors.

Figure 8. Air pollution tolerance index (APTI) of six plant species.

Examination of the Effectiveness of Selected Plant Species for Removing Atmospheric Ammonia

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Introduction

- According to the most recent statistics from the U.S. Department of Agriculture, Texas ranked 6th in broiler production in 2015.
- Consequently, the release of ammonia becomes one of the top-priority issues in CAFOs as it is one of the most generated waste by-products in livestock production.
- Technologies have been developed and adopted to mitigate and reduce the environmental impact of ammonia emissions from animal facilities but limitations arise because of issues on cost-effectiveness and performance of such technologies.
- In response to this, trees and plants have been considered as an emerging option as ammonia control measures by placing them strategically around CAFOs as they act as 'natural pollutant sink' intercepting dust and other pollutants.

Objectives

The overall objective was to examine the effectiveness of plants in the removal of atmospheric NH_3. Specifically, this study:
- Designed and assessed the performance of a static exposure chamber for measuring gaseous uptake by plants;
- Developed a modified method to quantify the concentration of foliar NH_3;
- Compared the foliar NH_3 content of six plant species; and
- Evaluated the effect of atmospheric NH_3 on the leaf gas exchange rate of six plant species.

Methods
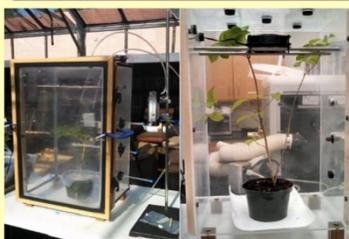
Exposure to Ammonia. Seedlings of Arborvitae, American holly, Arizona cypress, Roughleaf dogwood, Eastern red cedar and Yaupon (Figure 1) were exposed to 3 levels (1, 5, 10 ppm) of NH_3 in a controlled chamber housed in the Forestry Greenhouse.

Physiological measurement. An open design of gas exchange system (Model 6400, LI-COR Instruments, Lincoln, NE) was used to determine the effect of ammonia on the physiological responses of plants (Figure 2). Net photosynthetic rates, stomatal conductance and transpiration rate were obtained before and immediately after exposure to ammonia.
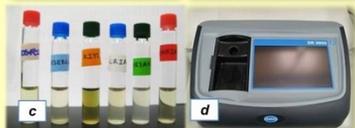
Foliar Ammonia Quantification. Randomly collected fresh plant foliage from each species were subjected to laboratory analysis for foliar ammonia content through a modified enzymatic technique at the Environmental Assessment Laboratory [Figure 3 (a),(b),(c),(d)].

Results and Discussions

- Results showed a significant statistical difference on the foliar ammonia content ($p<0.0001$). Generally, the trend is as follows : Eastern red cedar > American holly > Arizona cypress > Roughleaf dogwood > Arborvitae > Yaupon (Figure 4).
- Net photosynthetic rate (Figure 5), stomatal conductance (Figure 6) and transpiration rate (Figure 7) were all significantly influenced by the interaction of either two or all three factors (Species vs. Treatment levels vs. Exposure).

Conclusions

- The designed static exposure chamber was effective for short-term exposure study.
- The modified method of enzymatic technique was successfully used in quantifying the concentration of NH_3 absorbed by the leaves.
- Based on NH_3 assimilation, Eastern red cedar will be the best option for shelterbelt design followed by Arizona cypress, American holly, Roughleaf dogwood, Arborvitae and Yaupon.
- Exposure to NH_3 significantly lowered the net photosynthetic rates of the three plants analyzed with higher NH_3 content (Eastern red cedar, Arizona cypress and American holly).
- Exposure to NH_3 significantly increased the stomatal conductance and transpiration rate of Arizona cypress and American holly.
- All six plant species can be used as bio-indicators since they were all classified as sensitive (APTI II) to pollution.

Recommendations

- Measure the wall effect losses in future experiments.
- Increase the flow rate and extend the duration of exposure to reach the desired concentration and establish a stabilization period.
- Determine the compensation point for each plant species.
- Design a dynamic chamber system that will measure the actual NH_3 flux to model atmospheric-biospheric interactions.
- Determine if plants differ on their extinction rates based on their foliar characteristics.

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